STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER:

PTC923-PKU-301

STUDY TITLE:

A Phase 3, Randomized, Crossover, Open-Label, Active-Controlled Study of Sepiapterin versus Sapropterin in Participants with Phenylketonuria ≥2 years of Age

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialized Term	Explanation
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
BH_4	Tetrahydrobiopterin
BMI	Body mass index
BUN	Blood urea nitrogen
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CTCAE	Common Terminology Criteria for Adverse Events
DBS	Dried blood sample
ECG	Electrocardiogram
EOS	End of Study
EQ-5D	European Quality of Life - 5 Dimensions
EQ-5D-5L	5-Level European Quality of Life - 5 Dimensions
EQ-5D-Y	European Quality of Life - 5 Dimensions for Youth
ETV	Early Termination Visit
FAS	Full Analysis Set
GGT	Gamma glutamyl transferase
hCG	Human chorionic gonadotrophin
HEENT	Head, eyes, ears, nose, and throat
LDH	Lactate dehydrogenase
LS	Least square
MAR	Missing at random
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed model repeated measures model
MNAR	Missing not at random
PAH	Phenylalanine hydroxylase
Phe	Phenylalanine
PK	Pharmacokinetics
PKU	Phenylketonuria
PKU-QOL	Phenylketonuria -Quality of Life
PP	Per-Protocol
PT	Preferred Term
QOL	Quality of Life
RBC	Red blood cell
SAE	Serious adverse event
SAS	Statistical Analysis System
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
Tyr	Tyrosine
ULN	Upper Limit of Normal
VAMS	Volumetric Absorptive MicroSampling
WBC	White blood cell

1. INTRODUCTION AND OVERVIEW

The purpose of this statistical analysis plan is to describe the procedures and the statistical methods to support Study PTC923-PKU-301 final reporting after database lock. The statistical methods described here are based on the protocol issued on 06 Sep 2024, Version 4.0.

1.1. Study Design

This is a Phase 3, 2-part, randomized, crossover, open-label, active-controlled study of sepiapterin versus sapropterin in participants with PKU ≥2 years of age. Part 1 is an open-label sepiapterin responsiveness test and Part 2 is a randomized, active-controlled, open-label, crossover treatment period.

Approximately 100 participants will be enrolled in Part 1 (Open-Label Responsiveness Test) to achieve 42 participants randomized into Part 2 (Active-Controlled, Open-Label, Crossover Treatment Period).

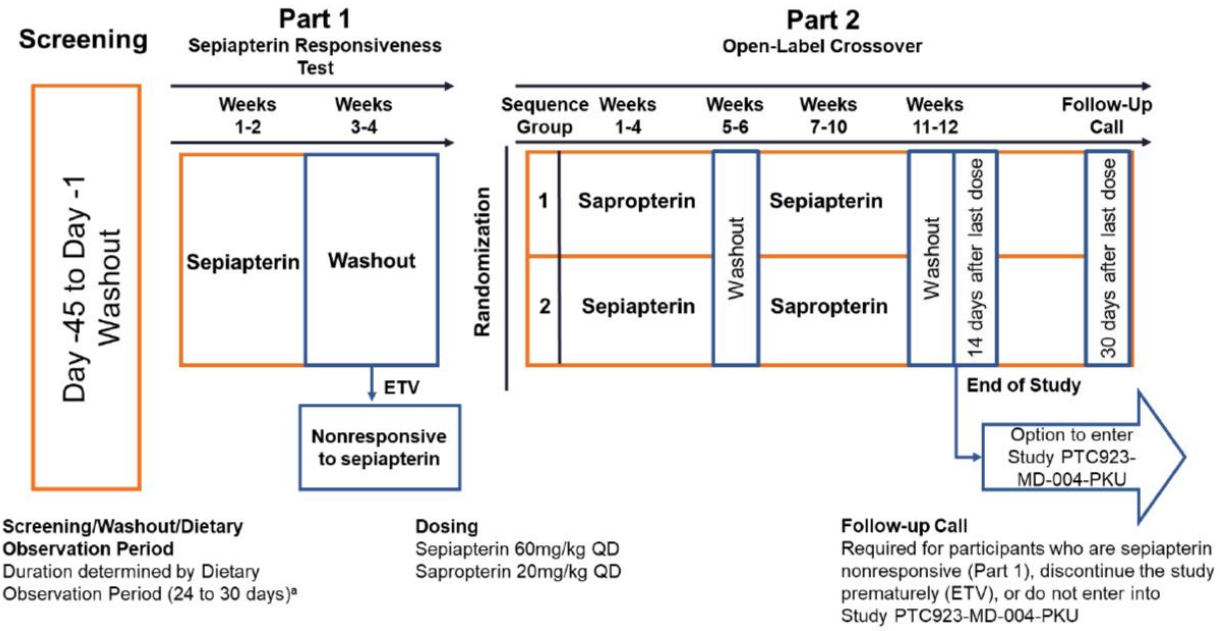
The screening period is up to 45 days. Participants who enter the study and are receiving BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) at the Screening Visit must complete a 7-day washout period prior to dosing.

Part 1 of the study tests for responsiveness to sepiapterin, with 14 days of open-label treatment with sepiapterin 60 mg/kg administered orally once a day followed by a minimum of 14 days sepiapterin washout. Blood Phe levels will be measured on Days -1 (only 1 sample is required if this is on the same day as BH_4 washout period Day 7), 1 (predose), 7, 10, and 14 of Part 1. Baseline blood Phe level will be calculated as the mean of the blood Phe levels from dried blood samples taken on Day -1 and Day 1 (predose). Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint. Following completion of 14 days of sepiapterin open-label treatment, participants will begin a minimum 14-day washout. Blood Phe levels will be measured on Days 19, 24, and 28 during the sepiapterin washout. During the sepiapterin washout, the mean change in blood Phe levels over the 14 days of sepiapterin treatment (mean of Part 1 Days 7, 10, and 14) will be measured and compared against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 [predose]). Participants who experience <20% reduction in blood Phe levels will be classified as non-responsive and will be contacted to schedule an Early Termination Visit (ETV) (on or between Part 1 Day 28 and Day 35).

On Part 2 Treatment Period (TP) 1 Day -1, all eligible participants will be randomized 1:1 to 1 of 2 treatment sequences (60 mg/kg sepiapterin-20 mg/kg sapropterin OR 20 mg/kg sapropterin-60 mg/kg sepiapterin). The randomization will be stratified based on their mean percent reduction in blood Phe levels from Part 1 (ie, participants with mean % reduction in Phe levels of ≥20% to <30% and participants with mean % reduction in Phe levels of ≥30%). Participants will receive each open-label treatment for 4 weeks (TP1 and TP2). Following completion of each 4-week treatment period, participants will complete a washout (minimum 14 days [+3 days window]).

For all participants who complete Part 2, End of Study (EOS) is defined as their final study visit on completion of the 14-day washout (+3 days) after Treatment Period 2. All participants who complete Part 2 will be given the opportunity to continue treatment in the sepiapterin open-label long-term study, PTC923-MD-004-PKU. The study design is summarized in Figure 1 and the Schedule of Assessments is presented in Table 3 (Screening Period) and Table 4 (Parts 1 and 2).

Figure 1: Study Schema for Study PTC923-301-PKU



Abbreviations: ETV, Early Termination Visit
[a] Participants with a >20% variance in dietary Phe consumption will be considered screen failures.
Note: Phe responsiveness is defined as mean Phe reduction ≥20%.

1.2. Study Objectives

1.2.1. Primary Objective

- To compare the efficacy of sepiapterin to sapropterin in reducing blood Phe levels in participants with PKU

1.2.2. Secondary Objectives

- To evaluate the efficacy of sepiapterin in reducing blood Phe levels
- To assess the safety and tolerability of sepiapterin

1.2.3. Exploratory Objective

- To evaluate changes in blood Tyr over time, including the Phe:Tyr ratio
- To assess the taste, palatability, and acceptability (<18 years) of sepiapterin
- To evaluate sepiapterin effect on QOL using the PKU-QOL questionnaire in the subset of participants who are able to complete the PKU-QOL (ie, participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years Parent PKU-QOL, ages 9 to 11 years Child PKU-QOL, ages 12 to 17 years Adolescent PKU-QOL, ages ≥18 years Adult PKU-QOL)
- To evaluate sepiapterin effect of QOL using the EQ-5D (EQ-5D-Y Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; EQ-5D-5L ([≥16 years])

1.3. Study Endpoints

1.3.1. Primary Endpoint

The primary endpoint is the mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period (the average of the last 2 weeks of each treatment period) in Part 2.

For Part 2 Treatment Period 1, baseline is defined as the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 1. For Part 2 Treatment Period 2, baseline is defined as the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 2. Mean level at Weeks 3 and 4 is calculated as the average of blood Phe concentration collected during the Week 3-4 analysis visit window of each treatment period as described in Section 3.4.4.

1.3.2. Secondary Endpoints

Proportion of participants with baseline blood Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L after each treatment period in Part 2

Phe level after each treatment period is referring to the average of blood Phe concentration collected during the Week 3-4 analysis visit window of each treatment period as described in Section 3.4.4. The binary endpoint will be calculated for all participants with baseline Phe levels ≥600 µmol/L:

- "Achieved" if Phe levels <600 µmol/L at the end of the treatment period
- "Not Achieved" if Phe levels still ≥600 µmol/L at the end of the treatment period

Proportion of participants reaching blood Phe <360 µmol/L after each treatment period in Part 2

The binary endpoint will be calculated for all participants with baseline Phe levels ≥360 µmol/L:

- "Achieved" if Phe levels <360 µmol/L at the end of the treatment period
- "Not Achieved" if Phe levels still ≥360 µmol/L at the end of the treatment period

Proportion of participants reaching blood Phe <120 µmol/L after each treatment period in Part 2

The binary endpoint will be calculated for all participants with baseline Phe levels ≥120 µmol/L:

- "Achieved" if Phe levels <120 µmol/L at the end of the treatment period
- "Not Achieved" if Phe levels still ≥120 µmol/L at the end of the treatment period

AEs, physical examinations, vital sign assessments, 12-lead ECGs, and routine clinical laboratory assessments

Safety and tolerability of sepiapterin will be evaluated by severity and number of treatment-emergent adverse events (TEAEs), clinical laboratory tests, vital signs, physical examinations, and electrocardiograms (ECGs) over the treatment period.

1.3.3. Exploratory Endpoints

The exploratory endpoints include:

- Mean change and percent change from baseline in blood Tyr and Phe:Tyr ratio over time
- Taste, palatability, and acceptability scores (participants <18 years)
- Changes from baseline in QOL using PKU-QOL questionnaire in the subset of participants that are able to complete the PKU-QOL (ie, participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years Parent PKUQOL, ages 9 to 11 years Child PKU-QOL, ages 12 to 17 years Adolescent PKUQOL, ages ≥18 years Adult PKU-QOL)
- Changes from baseline in QOL using the EQ-5D (EQ-5D-Y Proxy Version 1 [3 to7 years]; EQ-5D-Y [8 to 15 years]; EQ-5D-5L ([≥16 years])

1.4. Sample Size

For the primary efficacy endpoint in the stratum who achieve a ≥30% reduction in blood Phe levels in Part 1, mean changes in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period in Part 2, 34 participants (17 in each sequence group) will provide 90% power to detect a treatment difference between sepiapterin and sapropterin using a 2-group t-test (crossover analysis of variance) with a 2-sided 5% significance level, assuming a treatment difference of 115 μmol/L and a within-participant standard deviation of 200 μmol/L (assumption based on data from study PKU-002). The sample size calculation was performed by using software nQuery v9.2.1.0.

With an assumption of 20% attrition rate, the study will require 42 participants randomized into Part 2. Approximately 100 participants will be screened in Part 1 (Open-Label Responsiveness Test) to achieve 42 participants randomized into Part 2 (Active-Controlled, Open-Label, Crossover Treatment Period).

1.5. Randomization

In Part 1 of the study, all participants will receive open-label treatment with sepiapterin administered orally once a day.

Following the minimum 14-day sepiapterin washout period, all eligible participants will proceed to Part 2 and be randomized 1:1 to 1 of 2 treatment sequences (sepiapterin-sapropterin OR sapropterin-sepiapterin).

Part 2 of this study will be performed in an open-label fashion. Participants will be randomized 1:1 to 1 of 2 treatment sequences (sepiapterin-sapropterin OR sapropterin-sepiapterin) using a central randomization process. The randomization will be stratified based on their mean percent reduction in blood Phe levels from Part 1 (ie, participants with mean % reduction in Phe levels of ≥20% to <30% and participants with mean % reduction in Phe levels of ≥30%) and evenly distributed to either 1 of 2 treatment sequences (sepiapterin-sapropterin OR sapropterin-sepiapterin).

2. ANALYSIS SETS

Subject inclusion into each population for the final analysis will be determined prior to database lock.

2.1. Primary Analysis Set

Primary Analysis Set (PAS): All participants who achieved a ≥30% reduction in blood Phe concentrations in Part 1, are randomized and take at least 1 dose of study drug in Part 2 will be included in the PAS. Data will be analyzed according to the treatment sequence participants were randomized to. All efficacy analyses will be performed on the PAS.

2.2. Full Analysis Set

Full Analysis Set (FAS): All participants who are randomized and take at least 1 dose of study drug in Part 2 will be included in the FAS. Data will be analyzed according to the treatment sequence participants were randomized to. All efficacy analyses will be performed on the FAS.

2.3. Per-Protocol Analysis Set

The Per-Protocol (PP) analysis set will include all participants in the FAS who meet the study eligibility requirements and have no major protocol deviations that affect the validity of the efficacy measurements. PP is a subset of the FAS. Participants who meet the following criteria will be excluded from the PP population:

- Received study treatment different from the randomized treatment throughout Part 2
- Did not have a valid blood Phe at baseline of each period in Part 2, or a valid blood Phe at Week 3 - 4 of each period in Part 2 within the allowed analysis visit window
- Had significant non-compliance to study drug administration
- Had significant inclusion or exclusion criteria violations
- Had major protocol deviations (eg, diet modification) which may impact effectiveness of study treatment

The PP Analysis Set will be used for sensitivity analysis of the primary efficacy endpoint. A separate documentation containing the final exclusion criteria and a list of participants in this population will be finalized prior to database lock.

2.4. Safety Analysis Set

Safety Analysis Set will include all subjects who receive at least 1 dose of study drug, including during Part 1.

Furthermore, Safety Analyses Set for Part 2 will include all subjects who are randomized and take at least 1 dose of study drug in Part 2. Safety data will be analyzed according to actual treatment participants received at the time. For the actual treatment definition, if a subject received different treatment from randomized treatment throughout Part 2, that treatment would be actual treatment as opposed to the treatment the subject was allocated to receive at randomization.

3. GENERAL CONSIDERATIONS

3.1. Estimands

The primary estimand of the study is intended to provide a population level estimate of the treatment difference in terms of mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period in Part 2 by a treatment policy strategy, as summarized in Table 1.

Table 1: Primary Estimand of the Study

Endpoint	Population	Intercurrent Events	Population Level Summary
Mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period in Part 2	PKU participants with a reduction in blood Phe levels of ≥30% in Part 1	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and 95% confidence interval (CI) between sepiapterin and sapropterin in the mean change from baseline to Weeks 3 and 4 will be estimated using a mixed model repeated measures (MMRM) method.

3.2. Interim Analysis

No interim analysis is planned for this study.

3.3. Multicenter Study

It was estimated that subjects would be screened and enrolled across approximately 20 centers for this study. Due to limited data from each site, no adjustment or stratification for site will be performed. Data from all centers will be pooled for the primary analysis.

3.4. Data Definitions and Analysis Issues

3.4.1. Baseline Definitions

Baseline is defined for Part 1 and Part 2 respectively, unless specified otherwise.

For the analyses in Part 1, baseline is defined as the last available measurement prior to the first dose of sepiapterin during the Part 1 Open-Label period.

For the analysis in Part 2, baseline is defined as the last available measurement prior to the first dose of study drug (sepiapterin or sapropterin), during each treatment period in Part 2.

3.4.2. Blood Phe Data

The American College of Medical Genetics and Genomics guidelines recommend lifelong treatment of PKU, with the primary goal of therapy to maintain blood Phe <360 µmol/L (Smith 2025). As such, blood Phe levels are universally used in the diagnosis and clinical management of patients with hyperphenylalaninemia due to PKU. Blood Phe levels will be measured at the timepoints indicated in Table 4 and Table 5. Samples should be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day at each collection timepoint.

The main purpose of Part 1 of the study is to test for responsiveness to sepiapterin. In Part 1, blood Phe levels will be measured on Days -1, 1 (predose), 7, 10, and 14 of the treatment period,

and on Days 19, 24, and 28 during the washout period. The mean percent reduction in Phe level will be calculated as mean of Part 1 Days 7, 10, and 14 against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 predose). The calculated mean percent reduction in Phe level will be used to determine the responsiveness to sepiapterin. Phe responsiveness is defined as mean Phe reduction ≥20%.

Part 2, the randomized, open-label, crossover treatment period, is the main part of the study to evaluate the primary objective. Blood Phe levels will be measured in Part 2 on Days -1, 1 (predose) 7, 10, 14, 19, 24, and 28 for all subjects. Baseline is the mean of Day -1 and Day 1 predose blood Phe levels, and the mean blood Phe levels of every 2-week intervals will be calculated (Days 7, 10, and 14 for Weeks 1 and 2 and Days 19, 24, and 28 for Weeks 3 and 4) in Part 2. The mean change from baseline and percent change from baseline in blood Phe levels will be calculated for each of the 2-week treatment periods for each of the subjects.

Note that if there is one or more data points for blood Phe level missing (not taken, not evaluable, or lost) or VAMS filled with insufficient amount of blood for all the samples on any assessment day, then the remaining available data on other assessment day(s) during the calculation period will be used to calculate the mean value. Only when all the data points for blood Phe levels are missing, will the mean blood Phe level for that particular period be considered as missing.

3.4.3. Baseline Characteristics and Treatment Group Comparability

Baseline subject characteristics will be collected prior to Part 1 dosing for the overall Safety Analysis Set. Subjects who experience a <20% reduction in blood Phe levels will be classified as nonresponsive and be contacted to schedule an ETV. Accordingly, the baseline characteristics will be summarized by treatment group (including subjects that only participated in Part 1 as a treatment group) and by the Phe reduction randomization strata (≥20% to <30% or ≥30%, if participated in Part 2) using frequency for categorical variables and descriptive statistics for quantitative variables.

During the study, several levels of treatment groups will be used to summarize data:

- For the Safety Analysis Set, as all subjects who receive at least 1 dose of study drug in Part 1 will be included, the overall group represents all subjects treated in Part 1, but for display purposes, the treatment groups can be split into subjects who only participated in Part 1 and subjects who are randomized and treated in Part 2 (FAS), who can be further separated into (sepiapterin - sapropterin) or (sapropterin - sepiapterin) treatment groups. Subjects who only participated in Part 1 non-responders (subjects experience a <20% reduction in blood Phe levels), early termination subjects during Part 1, and subjects who were randomized but did not take any study drug during Part 2.
- For the PAS and FAS, the treatment groups can be composed of (sepiapterin – sapropterin) or (sapropterin – sepiapterin), and the overall includes all subjects randomized and treated in Part 2. Baseline characteristics tables will be further summarized by the Phe reduction randomization strata (≥20% to <30% or ≥30%) for the subjects who are randomized and treated in Part 2.

3.4.4. Study Analysis Visit

Study analysis visits during the study will be derived as listed in Table 2, based on study days from first dosing date during each Treatment Period (TP). Study analysis visits will be used in all by-visit summaries for both efficacy and safety assessments wherever appropriate.

Table 2: Study Analysis Visit

Analysis Visit	Scheduled Study Day	Analysis Window (in Study Days)
Screening		-45 days to Part 1 Day -2
Part 1 Day -1	Part 1 Day -1	Part 1 Day -1
Part 1 Day 1	Part 1 Day 1	Part 1 Day 1
Part 1 Day 2	Part 1 Day 2	Part 1 Day [2, 3]
Part 1 Day 7	Part 1 Day 7	Part 1 Day [4, 8]
Part 1 Day 10	Part 1 Day 10	Part 1 Day [9, 12]
Part 1 Day 14	Part 1 Day 14	Part 1 Day [13, 17]
Washout Day 19	Part 1 Day 19	Part 1 Day [18, 22]
Washout Day 24	Part 1 Day 24	Part 1 Day [23, 26]
Washout Day 28	Part 1 Day 28	Part 1 Day [27, last day in Part 1]
Part 2 TP 1 Day -1	Part 2 TP 1 Day -1	Part 2 TP 1 Day -1
Part 2 TP 1 Day 1	Part 2 TP 1 Day 1	Part 2 TP 1 Day 1
Part 2 TP 1 Day 7	Part 2 TP 1 Day 7	Part 2 TP 1 Day [2, 8]
Part 2 TP 1 Day 10	Part 2 TP 1 Day 10	Part 2 TP 1 Day [9, 12]
Part 2 TP 1 Day 14	Part 2 TP 1 Day 14	Part 2 TP 1 Day [13, 17]
Part 2 TP 1 Day 19	Part 2 TP 1 Day 19	Part 2 TP 1 Day [18, 22]
Part 2 TP 1 Day 24	Part 2 TP 1 Day 24	Part 2 TP 1 Day [23, 26]
Part 2 TP 1 Day 28	Part 2 TP 1 Day 28	Part 2 TP 1 Day [27, 31]
Part 2 TP 1 Week 1-2		Part 2 TP 1 Day [2, 17]
Part 2 TP 1 Week 3-4		Part 2 TP 1 Day [18, 31]
Part 2 TP 1 Washout Day 5	Part 2 TP 1 Day 33	Part 2 TP 1 Day [32, 35]
Part 2 TP 1 Washout Day 10	Part 2 TP 1 Day 38	Part 2 TP 1 Day [36, 40]
Part 2 TP 1 Washout Day 14	Part 2 TP 1 Day 42	Part 2 TP 1 Day [41], the date before Part 2 TP 2 Day -1
Part 2 TP 2 Day -1	Part 2 TP 2 Day -1	Part 2 TP 2 Day -1
Part 2 TP 2 Day 1	Part 2 TP 2 Day 1	Part 2 TP 2 Day 1
Part 2 TP 2 Day 7	Part 2 TP 2 Day 7	Part 2 TP 2 Day [2, 8]
Part 2 TP 2 Day 10	Part 2 TP 2 Day 10	Part 2 TP 2 Day [9, 12]
Part 2 TP 2 Day 14	Part 2 TP 2 Day 14	Part 2 TP 2 Day [13, 17]
Part 2 TP 2 Day 19	Part 2 TP 2 Day 19	Part 2 TP 2 Day [18, 22]
Part 2 TP 2 Day 24	Part 2 TP 2 Day 24	Part 2 TP 2 Day [23, 26]
Part 2 TP 2 Day 28	Part 2 TP 2 Day 28	Part 2 TP 2 Day [27, 31]
Part 2 TP 2 Week 1-2		Part 2 TP 2 Day [2, 17]
Part 2 TP 2 Week 3-4		Part 2 TP 2 Day [18, 31]
Part 2 TP 2 Washout Day 5	Part 2 TP 2 Day 33	Part 2 TP 2 Day [32, 35]
Part 2 TP 2 Washout Day 10	Part 2 TP 2 Day 38	Part 2 TP 2 Day [36, 40]
Part 2 TP 2 Washout Day 14	Part 2 TP 2 Day 42	Part 2 TP 2 Day [41, 14 days after the last dose in Part 2 TP 2]

For a given subject, if multiple assessments are within the same analysis window, the one closest to the scheduled study day and with non-missing value will be used for that analysis visit. In case of equal number of days to the scheduled visit date, the later assessment with non-missing value will be used for that given analysis visit. The assessment to be used is determined by using the following hierarchy (in decreasing order):

1) The assessment with non-missing value

2) The assessment closest to the target study day

3) The latter assessment, if ≥2 observations are equally close to the target study day

3.4.5. Multiplicity Control

To control the family-wise error rate, a gatekeeping procedure will be used.

First, the primary analysis of the mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period in Part 2 will be performed on the PAS, at the significance level of 0.05 (2-sided). If $p<0.05$, the study will be declared successful.

Only if the primary analysis is statistically significant, a test of the same endpoint based on the FAS and analyses of the secondary endpoints will be performed in the following order:

- Mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period in Part 2, based on the FAS
- Proportion of participants with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L after each treatment period in Part 2, based on the PAS
- Proportion of participants with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L after each treatment period in Part 2, based on the PAS
- Proportion of participants with baseline Phe levels ≥120 µmol/L who achieve Phe levels <120 µmol/L after each treatment period in Part 2, based on the PAS
- Proportion of participants with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L after each treatment period in Part 2, based on the FAS
- Proportion of participants with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L after each treatment period in Part 2, based on the FAS
- Proportion of participants with baseline Phe levels ≥120 µmol/L who achieve Phe levels <120 µmol/L after each treatment period in Part 2, based on the FAS

3.5. Changes to Protocol Specified Analysis

Change	Description
1	An additional secondary endpoint (Proportion of participants with baseline Phe levels ≥120 µmol/L who achieve Phe levels <120 µmol/L after each treatment period in Part 2), corresponding multiplicity control and analysis method are added.

4. PATIENT DATA

All subjects in the Safety Analysis Set will be included in the analysis by treatment groups (including subject that only participated in Part 1 as a treatment group). For subjects participating in Part 2 of the study, data will be further presented by the Phe reduction randomization stratum (≥20% to <30% or ≥30%) on the FAS. Subjects will be analyzed according to the treatment group into which they were randomized.

4.1. Patient Disposition

The disposition of subjects, including the number of subjects screened, the number of subjects who received at least 1 dose of study drug in Part 1, the number of randomized subjects in Part 2, the number of randomized subjects who received at least 1 dose of study drug in Part 2, and the number of subjects who prematurely discontinue study drug during Part 1, during each treatment period in Part 2 (along with the reason for the premature termination), completed Part 2 and were eligible to continue in the sepiapterin open-label study (Study PTC923-MD-004-PKU) will be summarized by treatment sequence.

The number of subjects randomized will also be summarized by region, country, and site.

Screen failures will be listed for each inclusion/exclusion criteria that were not met.

4.2. Data Sets Analyzed

A summary of the number of subjects for each analysis dataset will be provided by treatment groups.

The number of subjects with major protocol deviation will be summarized for both FAS and Safety populations.

4.3. Demographics and Baseline Characteristics

Demographics and background disease characteristics will be summarized with descriptive statistics by treatment sequence. Summary statistics for categorical variables will include frequency counts and percentages and for continuous variables will include mean, standard deviation, minimum, median, and maximum.

Demographics and baseline characteristics variables include:

- Age at screening (year) and by categories (2-5 years, 6-11 years, 12-18 years, ≥18 years) and Pediatric (<18 years, ≥18 years)
- Sex (Male, Female)
- Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, and Other)
- Screening weight
- Screening height
- Screening body mass index (BMI) calculated from weight and height

4.4. Disease Characteristics

PKU history and phenylalanine hydroxylase genotype information (including newly assessed or previously done) will be descriptively summarized by treatment sequence.

A summary will be provided by treatment sequence regarding if subjects have previously received treatment with BH_4 or pegvaliase-pqpz, if the subjects previously responded to BH_4 challenge, and if the subjects are receiving treatment with BH_4 or pegvaliase-pqpz at the time of screening. The data collected during the washout period (including the blood Phe data and 3-day diet records) will be summarized by treatment sequence.

Classical PKU will be summarized by treatment sequence.

4.5. Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA). Medical history information will be descriptively summarized by treatment sequence.

4.6. Prior and Concomitant Medications and Non-Drug Treatments

Any treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) taken by a subject 30 days prior to the Screening Visit and during the course of the study and the reason for use of the medication will be recorded in the electronic case report form (medications and prescribed dietary supplements). Data will be coded using the World Health Organization Drug Dictionary. Medication stopped before Part 1 Day 1 will be counted as prior medications, and medication that had not stopped by Part 1 Day 1 or started on or post Part 1 Day 1 will be reported as concomitant medications. Prior medications and concomitant medications will be summarized descriptively by treatment sequence.

Procedure data will be summarized descriptively by treatment sequence.

4.7. Extent of Exposure

The extent of exposure to study drug is defined as the last dose date minus the first dose date +1 day within each part (Part 1, Part 2 TP 1, and Part 2 TP 2) of the study. Data will be summarized by treatment group (sepiapterin or sapropterin) for each part of the study.

The overall extent of exposure is the sum of the exposure in both parts of the study.

4.8. Treatment Compliance

Treatment compliance will be assessed by the amount of total dose actually taken divided by the amount of total dose that should have been taken.

$$Compliance\ (\%) = \frac{Amount\ of\ total\ dose\ actually\ taken\ during\ the\ treatment\ period}{Expected\ amount\ of\ total\ dose\ during\ the\ treatment\ period} \times 100$$

Data will be summarized descriptively by part and by treatment group.

5. EFFICACY ANALYSIS

5.1. Primary Endpoint Analysis

The primary efficacy endpoint is the mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period (the average of the last 2 weeks of each treatment period) in Part 2.

The null hypothesis is that the mean change in blood Phe levels from baseline to Weeks 3 and 4 is the same between sepiapterin and sapropterin, versus the alternative that they are different, at the 2-sided 0.05 significance level.

A mixed model repeated measures (MMRM) model will be fitted on the calculated mean change in blood Phe from baseline to Weeks 3 and 4 of each treatment period in Part 2 for each participant, based on the testing procedure in section 3.4.5. The model will include fixed effects for treatment group, sequence, period, visit (Weeks 1 - 2 and Weeks 3 - 4), and treatment-by-visit interaction, and the baseline blood Phe (for each period) as a covariate. In addition, participant nested within sequence will be included as a random effect. The MMRM model will be used on available data assuming the missing assessments are missing at random (MAR), no explicit imputation is involved. An unstructured within-subject covariance structure will be assumed. If the model does not converge under the unstructured covariance matrix, the following covariance structures will be employed in order until convergence is reached: heterogeneous Toeplitz, heterogeneous Compound Symmetry, heterogeneous first-order autoregressive, Toeplitz, Compound Symmetry, and first-order autoregressive. The least squares (LS) mean estimate for the change in blood Phe levels from baseline to Weeks 3 and 4 will be used to perform treatment group comparisons. The LS means, treatment effect estimate, 95% confidence interval, and 2-sided p-value will be presented. The SAS pseudocode code is provided in Appendix 2.

For the analysis on FAS, an additional fixed effect term - the randomization stratum (mean % reduction in Phe levels of ≥20% to <30% or ≥30% in Part 1) will be included in the model.

5.2. Sensitivity Analysis

5.2.1. Analysis on PP Analysis Set

The above MMRM model for the primary endpoint on FAS in Section 5.1 will also be performed on the PP Analysis Set.

5.2.2. Completer analysis with Analysis of Covariance (ANCOVA) model

For this sensitivity analysis, only the completers who have the assessments at baseline and Weeks 3-4 in each period of Part 2 will be included in the ANCOVA model. For the analysis on PAS, the model will include fixed effects for treatment group, sequence, and period; the baseline blood Phe (for each period) will be used as a covariate. The randomization stratum will also be added to the analysis on FAS.

5.2.3. Multiple imputation

To evaluate the MAR assumption in the primary analysis on both PAS and FAS, a pattern-mixture model approach to multiple imputation (MI) under the missing not at random (MNAR)

assumption by creating control-based pattern imputation will be used. For participants in the sepiapterin group who discontinued early, their response distribution after discontinuation would follow the distribution in the control group. The procedure will only be implemented by each period if at least 10% missing assessments (ie, ≥2 participants) at Weeks 3 and 4 in that period in Part 2. The SAS pseudocode code is provided in Appendix 2.

5.3. Subgroup Analysis

The change in blood Phe levels from baseline to Weeks 3 and 4 will be summarized in the following subgroups, for both PAS and FAS:

- Gender (Male, Female)
- Age:
  - <18 years, ≥18 years
  - 2 to 5 years, 6 to 11 years, 12 to 18 years, ≥18 years
- Respond to BH_4 challenge (Yes, No)
- Subjects who enter the study and were receiving supplementation treatment (pegvaliase-pqpz, BH_4, or none)
- Classical PKU (Yes, No)
- Baseline Phe level of Part 2
  - ≥600 µmol/L, <600 µmol/L
  - ≥360 µmol/L, <360 µmol/L
  - ≥120 µmol/L, <120 µmol/L

For the FAS dataset, one additional subgroup analysis will be performed:

- Phe reduction in Part 1 (≥20% to <30%, ≥30%).

5.4. Secondary Endpoints Analysis

Blood Phe levels are collected at the timepoints indicated in Table 3, Table 4, Table 5, and Table 6. The concentration data in Part 1 (collected on Days -1, 1 (predose), 7, 10, and 14 of the treatment period and on Days 19, 24, and 28 during the washout period), the mean baseline (mean of Day -1 and Day 1 predose), the mean 2-week treatment (mean of Part 1 Days 7, 10, and 14) data will be descriptively summarized by treatment group. The mean change and percent change from baseline will also be descriptively summarized. The following categories (reduction <20%, ≥20% to <30% or ≥30%, value <360 µmol/L or ≥360 µmol/L, and value <600 µmol/L and ≥600 µmol/L at baseline or post-treatment period) will be summarized with number of events and percentage by treatment group.

The concentration data in Part 2 (collected on Days -1, 1 (predose), 7, 10, 14, 19, 24, 28, Washout Days 5, 10, and 14), the mean baseline (mean of Day -1 and Day 1 predose), the mean of each 2-week treatment of each dose level (the average blood Phe levels of Day 7, 10 and 14 is for the 2-week period of Week 1-2, Day 19, 24 and 28 is for the 2-week period of Week 3-4, and Washout Days 5, 10, and 14 is for the 2-week period of washout Week 5-6) will be descriptively

summarized by treatment group, along with the mean change and percent change from baseline in Part 2. The following categories (reduction <20%, ≥20% to <30% or ≥30%, value <360 µmol/L or ≥360 µmol/L, and value <600 µmol/L and ≥600 µmol/L at baseline or post each 2-week treatment periods) will be summarized with number of events and percentage by treatment group.

Graphs will be generated to display the mean concentration data over time by treatment groups.

Proportion of participants with baseline Phe Levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of each treatment period

A binary variable will be defined as achieved versus not-achieved, for participants with baseline blood Phe levels ≥600 µmol/L, whether they achieve Phe levels <600 µmol/L with an average of blood Phe concentration collected during the Week 3-4 analysis visit window of each treatment period. The data will be analyzed by a mixed-effect logistic regression model based on the PAS. The model will include fixed effects for treatment group, sequence, period, and the baseline blood Phe (for each period) as a covariate. In addition, participant nested within sequence will be included as a random effect. The odds ratio of sepiapterin versus sapropterin along with the 95% CI will be presented.

For the analysis on FAS, an additional fixed effect term - the randomization stratum (mean % reduction in Phe levels of ≥20% to <30% or ≥30% in Part 1) will be included in the model.

Proportion of participants with baseline Phe Levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of each treatment period

A binary variable will be defined as achieved versus not-achieved, for participants with baseline blood Phe levels ≥360 µmol/L, whether they achieve Phe levels <360 µmol/L with an average of blood Phe concentration collected during the Week 3-4 analysis visit window of each treatment period. The same model as above for PAS and FAS will be used for the analysis of this endpoint. The odds ratio of sepiapterin versus sapropterin along with the 95% CI will be presented.

Proportion of participants with baseline Phe Levels ≥120 µmol/L who achieve Phe levels <120 µmol/L at the end of each treatment period

A binary variable will be defined as achieved versus not-achieved, for participants with baseline blood Phe levels ≥120 µmol/L, whether they achieve Phe levels <120 µmol/L with an average of blood Phe concentration collected during the Week 3-4 analysis visit window of each treatment period. The same model as above for PAS and FAS will be used for the analysis of this endpoint. The odds ratio of sepiapterin versus sapropterin along with the 95% CI will be presented.

5.5. Exploratory Endpoints

Descriptive statistics will be used for all the exploratory endpoints.

Tyrosine Concentrations

Blood Tyr levels are collected at the same timepoints as blood Phe as indicated in Table 4 and Table 5. The Part 1 and Part 2 Tyr concentration data and the Phe:Tyr ratio at each timepoint will be summarized similarly to Phe concentration data, and the same way of calculating the mean baseline and mean 2-week treatment period data, along with mean change and percent change from mean baseline. Graphical illustration will be provided similarly as blood Phe data.

Taste, palatability, and acceptability scores

On receipt of first dose of sepiapterin in Part 1 (responsiveness test), all participants <18 years of age will complete taste, palatability, and acceptability assessments.

Taste and palatability

For participants <5 years of age: palatability will be indirectly assessed by the parent(s)/caregiver(s) of participants using the following: On the basis of the reaction/facial expression of your child, do you think that the medication is (pleasant, not sure, unpleasant)?

For participants ≥5 to <18 years of age who are able to comply with the instructions: the participant will rate the taste/favor using a facial hedonic scale (5=really good; 4=good; 3=not sure; 2=bad; 1=really bad).

Acceptability

For all participants <12 years of age: parent(s)/caregiver(s) will also rate the acceptability/ease of administration, with the following question: Do you sometimes have problems in giving the medication to your child because they refuse to take it or throws it up? (Y/N)

Taste, palatability and acceptability will be summarized by corresponding age groups with frequency counts.

Phenylketonuria Quality of Life Questionnaire

In this study, a PKU-specific version of the quality of life (QOL) questionnaire will be performed at Part 2 Period 1 Day 1 (predose), Part 2 Period 1 Day 28, and Part 2 Period 2 Day 28. This will only be analyzed on FAS. Four versions of the Phenylketonuria Quality of Life Questionnaire (PKU-QOL) will be utilized dependent on subject age: Parent PKU-QOL (6 to 8 years old, 54 items), Child PKU-QOL (9 to 11 years old, 40 items), Adolescent PKU-QOL (12 to 17 years old, 58 items), and Adult PKU-QOL (≥18 years, 65 items). This will be conducted using the tool in one of the following validated languages: English (British or American), Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the PKU-QOL will not be required for subjects whose primary language is not one of the available validated languages.

The PKU-QOL questionnaire comprise of 4 modules: 1) PKU symptoms, 2) PKU in general, 3) administration of Phe-free protein supplements, and 4) dietary protein restriction. The recall period focuses on the past 1 week for all sections except for 'patient's general feeling' where the recall period was 'in general'.

The domain scores within the 4 modules will be calculated by the three-step scoring instruction described in the PKU-QoL manual (Mapi 2015) in a range from 0 to 100 for each of the four version questionnaires. The following interpretation rules were applied for all domain scores in a range from 0 to 100: for symptom scores, a higher score is associated with more frequent symptoms; for adherence scores, a higher score is associated with a poorer adherence; for other scores, a higher score is associated with a greater impact (Alptekin 2018). The domain scores will be summarized by age categories (corresponds to the 4 versions of the PKU-QOL) and treatment group.

The domain scores and change from baseline will be summarized by treatment group and by age category (corresponds to the 4 versions of the PKU-QOL) and visit for Full Analysis Set.

European Quality of Life – 5 Dimensions

The European QOL-5 dimensions (EQ-5D) questionnaire will be performed at Part 2 Day 1 (predose), Part 2 Day 28, and Part 2 Period 2 Day 28. In this study, 3 versions of the EQ-5D will be used: EQ-5D-Y Proxy Version 1 (3 to 7 years), EQ 5D-Y (8 to 15 years), and EQ-5D-5L (≥16 years) measuring 5 domains of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression (EuroQoL Research Foundation 2019, EuroQol Research Foundation 2020). Data will be summarized by EQ-5D versions and by treatment groups for index score which is derived based on country-based value set and each of the five health domains (refer to Appendix 3 for detail on deriving index score based on country-based value set).

For subjects aged ≥16 years, the EQ-5D-5L will be used. For the EQ-5D-5L, subjects will rate their own health on each dimension, with each dimension having 5 levels of severity. Dimensions include mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

For children/adolescents aged 8 to 15 years, the EQ-5D-Y will be used. This is a child friendly- version of the EQ-5D-5L. Dimensions include mobility, looking after myself, doing usual activities, having pain or discomfort, and feeling worried, sad, or happy. The younger subjects will be asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the 5 dimensions. The instructions and wording are more suitable for children and adolescents.

For children aged 3 to 7 years, a proxy version of the EQ-5D-Y will be used. This is for use when children or adolescents are mentally or physically incapable of reporting on their own health-related QOL. For a proxy version, a caregiver who knows the child or adolescent well will rate the health-related QOL in their opinion.

The index score and the domain scores and change from baseline will be summarized by treatment group and by age category and visit for Full Analysis Set.

6. SAFETY ANALYSES

All subjects in the Safety Analysis Set will be included in the analysis by assigned treatment within corresponding parts. Subjects will be analyzed according to actual treatment received.

6.1. Adverse Events

Adverse events (AEs) will be coded using MedDRA. The severity of AE will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (refer to the study manual).

Only those AEs that are treatment-emergent will be included in summary tables. Two sets of treatment-emergent AEs (TEAE) will be considered:

- TEAEs in Part 1 include all AEs occurring after first dose in Part 1 but before first dose in Part 2 for continuing subjects, or up to 30 days from the last dose for discontinued subjects.
- TEAEs in Part 2 include all AEs occurring after first randomized dose in Part 2 and up to 30 days from the last dose. Data will be presented by actual treatment at time of onset of the AE and overall. AEs that occur at or after the time of administration for a particular treatment and prior to the time of administration of next sequential treatment will be assigned to that particular treatment. AEs occurring during the washout periods will be assigned to the previous treatment period.

All AEs, treatment-emergent or otherwise, will be presented in subject data listings. An overview table of TEAEs, including number of subjects with TEAEs, treatment-emergent serious adverse events (SAEs), deaths, TEAEs classified as CTCAE Grade 3 or higher, study drug related TEAEs, TEAEs leading to study drug withdrawal will be provided for each treatment group. The following summaries will be produced for the TEAEs by treatment groups:

- Incidence of TEAEs by Preferred Term (PT) in descending order
- Incidence of TEAEs by System Organ Class (SOC) and PT
- Incidence of treatment-related TEAEs by SOC and PT
- Incidence of TEAEs by SOC, PT, maximum CTCAE grade
- Incidence of treatment-related TEAEs by SOC, PT, and maximum CTCAE grade

In the summary tables, subjects may be counted under multiple SOCs and PTs, but for each SOC and PT, subjects are only counted once. If a subject has the same AE on multiple occasions, the highest severity (5=fatal, 4=life-threatening, 3=severe, 2=moderate, 1=mild) recorded for the event will be presented and the highest drug relationship (1='Unrelated', 2='Unlikely to be Related', 3='Possibly Related', 4='Probably Related'), reclassified into Related ('Possibly Related', 'Probably Related') or Not Related ('Unrelated', 'Unlikely to be Related'), will be presented on the respective tables.

The following summaries will also be presented for the treatment-emergent SAEs:

- Incidence of treatment-emergent SAEs by SOC and PT
- Incidence of treatment-related treatment-emergent SAEs by SOC and PT

In addition, the number and percentage of subjects with TEAEs and treatment-related TEAEs leading to discontinuation from study treatment will also be summarized by SOC and PT for each treatment group.

6.2. Vital Signs and Weight

Vital signs will be collected at the Screening Visit, Part 1 Day 1, Days 1 and 28 in Part 2 in both periods, and Early Termination Visit (ETV) (if applicable). Vital signs will be collected both predose and 2 hours postdose on Part 1 Day 1, Part 2 TP1 Day 1, and Part 2 TP2 Day 1; for all other timepoints, they will be taken at any time during the visit prior to laboratory sampling.

Weight to be completed prior to initial dosing for Part 1 Day 1 and Part 2 TP1 Day 1, and Part 2 TP2 Day 1. Weight can be measured at any time during the other visits.

Height will be collected at the Screening Visit. Additionally, for participants <18 years of age, height should be measured at Part 2 TP1 Day 28 and TP2 Day 28, at the EOS Visit, and at any Unscheduled Visits when eGFR calculation is desired.

The vital signs assessments (including blood pressure, pulse, respiratory rate, and temperature) and weight (and BMI) will be descriptively summarized for all the specified timepoints by assigned treatments, including the change from corresponding baseline, for Part 1 and Part 2 separately.

6.3. Physical Examination

A full physical examination will be performed predose at the Screening Visit, Part 1 Day 1, Part 2 TP1 Day 1 and Day 28, Part 2 TP2 Day 1 and Day 28, and ETV (if applicable). General appearance, dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological, and skin parameters will be assessed.

Physical examination results will be listed in subject listings only.

6.4. Electrocardiogram

Twelve-lead ECGs will be obtained before any other study-related procedures are performed. If feasible, ECGs will be performed after a 5-minute rest in the supine position. Twelve-lead ECGs will be performed at Part 1 Day 1 (predose), Part 2 Day 28 for each treatment period, EOS for all participants, and at the ETV (if applicable). Electrocardiograms will be performed in triplicate, with each read taken at least 1 minute apart.

The ECG parameters (RR interval, PR interval, QRS interval, QT interval, and QTcF interval) and the overall interpretation of ECG (normal, abnormal clinically significant, or abnormal not clinically significant) will be descriptively summarized for all the specified timepoints by assigned treatments for Part 1 and Part 2 separately. For triplicate measurements, the mean of the three values will be used to summarize.

QTcF will be calculated with the following formular:

$$QTcF = \frac{QT}{\sqrt[3]{\frac{RR}{1\ sec}}}$$

Categorical analysis of the maximum post-baseline value in QTc interval will be performed to summarize the number and percentage of subjects meeting or exceeding the criteria below.

- Absolute QTcF interval prolongation:
  - - QTcF interval > 450
  - - QTcF interval > 480
  - - QTcF interval > 500
- Change from baseline in QTcF interval:
  - - QTcF interval increases from baseline >30
  - - QTcF interval increases from baseline >60

A listing of all abnormal values will be provided.

6.5. Central Laboratory Values

Blood and urine samples for clinical chemistry, hematology, and urinalysis will be collected at the timepoints in Table 2 and Table 3. Laboratory parameters that will be assessed will include, but not be limited to:

- Hematology: hematocrit, hemoglobin, platelet count, red blood cell count (RBC), white blood cell count (WBC), WBC differential
- Clinical chemistry: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, B12, blood urea nitrogen, calcium, CO_2, chloride, gamma glutamyl transpeptidase, glucose, iron, lactate dehydrogenase, phosphate, potassium, serum creatinine, sodium, total bilirubin, direct bilirubin, total cholesterol, total protein, and uric acid
- Urinalysis: bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen, microscopic examination of WBC, RBC, and epithelial cells
- Pregnancy testing: serum pregnancy test (human chorionic gonadotrophin) (Screening), urine pregnancy test (Parts 1 and 2)

Clinical laboratory test results and laboratory marked abnormalities using pre-defined abnormality criteria will be descriptively summarized for all the specified timepoints by assigned treatments, for Part 1 and Part 2 separately. In addition, shift tables on abnormality for each laboratory parameter from corresponding baseline to each of the post-treatment visit will be provided for Part 2.

Categorical analysis of the maximum post-baseline value in lab parameters of liver function test will be performed to summarize the number and percentage of subjects meeting or exceeding the criteria below, for Part 1 and Part 2 separately.

Category	Criteria
Elevated Aminotransferases	• ALT >3xULN • AST >3xULN • ALT or AST > 3 x ULN

Category	Criteria
Elevated Bilirubin	• Total Bilirubin >2xULN • ALT or AST >3xULN and Total Bilirubin >2xULN
Elevated Alkaline Phosphatase	• ALP >1.5xULN • ALT or AST >3xULN, Total Bilirubin >2xULN, and ALP >1.5xULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal

6.6. Contraception and Pregnancy Reporting

A listing will be provided.

6.7. Diet Monitoring: 3-Day Diet Records

Subjects will maintain 3-day diet records during the study as indicated in Table 3 and Table 4.

The 3-day diet records will be completed over 3 consecutive days within each period and collected weekly (Part 1) and biweekly (Part 2). The total Phe consumption during the 3-day period calculated by the dietician and daily total Phe consumption (mg/day) and daily Phe consumption (mg/kg/day) will be descriptively summarized by visit and by treatment group, including the change from baseline for Part 1 and Part 2 separately, where baseline will be calculated by averaging weekly 3-day diet records collected during the entire screening period. Graphical illustration will be provided.

7. MOCK TABLES, LISTINGS, AND GRAPHS

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

8. REVISION HISTORY

Version Number	Date	Description
1.0	31Mar2025	Initial release

APPENDIX 1. SCHEDULE OF ASSESSMENTS

Table 3: Schedule of Assessments (Screening Period and Part 1 [Open-Label Responsiveness Test])

Evaluation	Screening Period (Up to a Total of 45 Days)		Part 1 - Open-Label Responsiveness Test								ETV
	Screening Visit	BH_4 Washout Period	Sepiapterin Open-Label Treatment 60 mg/kg					Sepiapterin Washout			
	Up to 45 Days	7 Days[a]	14 Days					Min 14 Days			
Study Day			**-1**	**1**	**7**	**10**	**14**	**19**	**24**	**28[b]**	
Informed consent/assent	X										
PAH genotyping[c]	X										
Confirm eligibility	X									X	
Demographics	X										
Medical history[d]	X										
Enrollment				X							
In-clinic visit	X			X							X
Virtual visit							X			X[e]	
Vital signs[f], weight	X			X[g]							X
Height	X										
ECGs[h]				X[g]							X
Physical examination[i]	X			X[g]							X
Clinical laboratory tests[j]	X[k]			X[g]							X
Serum/urine pregnancy test[l]	X			X[g]							X
Concomitant medications[m]	Collected through the study										
Adverse events[n]	Collected through the study										
Blood Phe/Tyr levels (DBS)[o]	X	Day 1,4, 7	X	X	X	X	X	X	X	X	
Palatability assessments (<18 years only)[p]				X							
Consistent diet/diet monitoring/3-day diet record[q]	X[r]	X		X							
Dose study drug				Daily for 14 days							

Evaluation	Screening Period (Up to a Total of 45 Days)		Part 1 - Open-Label Responsiveness Test								ETV
	Screening Visit	BH_4 Washout Period	Sepiapterin Open-Label Treatment 60 mg/kg					Sepiapterin Washout			
	Up to 45 Days	7 Days[a]	14 Days					Min 14 Days			
Study Day			**-1**	**1**	**7**	**10**	**14**	**19**	**24**	**28[b]**	
Dispense study drug				X[s]							
Collect study drug, assess compliance							X[s]				X

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BH_4, tetrahydrobiopterin; BUN, blood urea nitrogen; COVID-19, coronavirus disease 2019; DBS, dried blood sample; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; EOS, End of Study; ETV, Early Termination Visit; GGT, gamma glutamyl transferase; HEENT, head, eyes, ears, nose, and throat; LDH, lactate dehydrogenase; PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU-QOL, Phenylketonuria-Quality of Life; RBC, red blood cell; SAE, serious adverse event; Tyr, tyrosine; WBC, white blood cells

[a] BH_4 treatment (eg, sapropterin dihydrochloride, KUVAN) will be discontinued 24 hours prior to starting the Supplementation Washout. Participants will remain off supplementation throughout the study.

[b] Visit can occur at any point on or between Day 28 and Day 35.

[c] To be performed unless documented in participant's medical history.

[d] Includes specific information related to any prior or existing medical conditions or surgical procedures involving the following systems: dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological, and skin. Newborn Phe concentrations and the 3 most recent Phe concentrations will be collected.

[e] Telephone call to confirm eligibility for continued participation and to schedule either Part 2 Day 1 visit or ETV.

[f] Includes blood pressure, pulse, respiratory rate, and temperature. Obtain vital signs prior to collection of any laboratory samples (including Phe). Vital signs should be collected prior to blood Phe/Tyr collection at the Screening Visit. If feasible, participants will rest for 5 minutes in a supine position before vital signs are assessed.

[g] To be completed prior to initial dosing and at additional timepoints where indicated (eg, 2-hour postdose vital signs).

[h] If feasible, participants will rest for 5 minutes in a supine position before ECG is performed. Twelve-lead ECGs will be performed at Part 1 Day 1 (predose) and at the ETV (if appropriate). Electrocardiograms will be performed in triplicate, with each read taken at least 1 minute apart.

[i] Conduct a complete physical examination of general appearance, dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological, and skin parameters.

[j] Includes clinical chemistry panel (albumin, AP, ALT, AST, B12, BUN, calcium, CO_2, chloride, serum creatinine, GGT, glucose, iron, LDH, phosphate, potassium, sodium, total bilirubin, direct bilirubin, total cholesterol, total protein, and uric acid), hematology panel (hematocrit, hemoglobin, platelet count, RBC, WBC, and WBC differential), and urinalysis (bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen and microscopic examination of WBC, RBC, and epithelial cells). Participants should be fasted prior to collection of samples (minimum of 4 hours for participants <6 years and minimum of 8 hours for participants ≥6 years).

[k] eGFR should be calculated for all participants.

[l] Pregnancy tests are required for all women of childbearing potential; serum pregnancy testing to occur during the initial Screening Visit and urine pregnancy testing to occur during all other in-clinic visits. Any positive urine pregnancy test should be confirmed by a serum pregnancy test performed at the site's local laboratory.

[m] Record all treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) starting from 30 days prior to Screening through the study. Specific information related to previous use of sapropterin or pegvaliase-pqpz should be collected (ie, details on duration, dose, if discontinued, the reason(s) why, and if a participant is known to be BH_4-responsive).

[n] Adverse events will be collected from the time of informed consent and/or assent (as applicable) until completion of EOS Visit or ETV (for participants who discontinue prematurely or are considered non-responsive to sepiapterin after Part 1, participants will continue to be followed for 30 days after last dose for SAEs).

[o] Blood Phe/Tyr levels via dried blood sample will be measured at each timepoint indicated. If BH_4 washout period Day 7 is the same day as Day -1 of Part 1, only 1 dried blood sample should be collected. Samples will be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day at each collection timepoint. Blood Phe/Tyr samples can be collected while the participant is either in the clinic or at home. Samples obtained at home will be shipped to the study site. Analysis of the screening blood Phe level can be performed at the site's local laboratory for determination of eligibility; however, a separate sample for blood Phe/Tyr should still be sent to the central laboratory for the screening timepoint. Vital signs should be collected prior to blood Phe/Tyr collection at the Screening Visit.

[p] Palatability assessments will be performed within 5 minutes of sepiapterin administration (see Section 8.4) in participants <18 years.

[q] Participants will maintain 3-day diet records during each week of Screening and Part 1, and a dietician will monitor each participant's diet to calculate total protein and corresponding Phe consumption, have regular contact with participants, and reinforce the need for participants to maintain their usual diet without modification.

[r] Participants with a >20% variance in dietary Phe consumption during the 30-day dietary control observation period will be considered screen failures.

[s] If feasible, study drug should be dispensed and returned at the study site. In the event that a participant cannot go into the study site (ie, due to COVID-19 restrictions, inability to make visit), study drug may be shipped directly to the participant's home/study site. In the event that a participant cannot go into the study site, unused study drug can be shipped directly back to the study site per local guidance.

An ETV will be performed on or between Day 28 and Day 35 for all participants who are determined to be non-responsive to sepiapterin in Part 1, or within 2 days for any participant who discontinues the study prematurely during Part 1 or Part 2 for any other reason. If eGFR calculation is desired, participants <18 years of age should have their height and serum creatinine measured, and participants ≥18 years of age should have their serum creatinine measured. Additionally, a phone follow-up visit will occur 30 (±3) days after the last dose of study drug to assess for SAEs.

If any unscheduled visits are performed when eGFR calculation is desired, alongside any assessments/procedures considered appropriate by the Investigator (or designee), participants <18 years of age should have their height and serum creatinine measured, and participants ≥18 years of age should have their serum creatinine measured.

Table 4: Schedule of Assessments (Part 2 [Randomized, Active-Controlled Treatment Period)

Evaluation	Part 2 - Randomized, Active-Controlled Open-Label, Crossover Treatment Period																			
	Treatment Period 1 Sepiapterin 60 mg/kg OR 20 mg/kg Sapropterin 28 Days (4 Weeks)								Washout Period 1 (14 Days Min)[a]	Treatment Period 2 Sepiapterin 60 mg/kg OR 20 mg/kg Sapropterin 28 Days (4 Weeks)								Washout Period 2 (14 Days Min)[a]	EOS[b]	ETV[c]
Study Day	**-1**	**1**	**7**	**10**	**14**	**19**	**24**	**28**	**1-14**	**-1**	**1**	**7**	**10**	**14**	**19**	**24**	**28**	**1-14**		
Randomization	X[d]																			
In-clinic visit		X[e]						X[e]			X[e]						X[e]		X	X
Virtual visit					X									X						
Vital signs[f],weight		X[g]						X			X[g]						X		X	X
Height[h]								X[c]									X[c]		X[c]	
ECGs[i]								X									X		X	X
Physical examination[j]		X[g]						X			X[g]						X		X	X
Clinical laboratory tests[k]		X[g]						X[g]			X[g]						X[l]		X[l]	X
Urine pregnancy test[m]		X[g]						X			X[g]						X		X	X
Concomitant medications[n]	Collected throughout the study																			
Adverse events[o]	Collected throughout the study																			
Blood Phe/Tyr levels (DBS)[p]	X	X	X	X	X	X	X	X	D5, 10, 14	X	X	X	X	X	X	X	X	D5, 10, 14	X	X
Consistent diet/ diet monitoring/3-day diet record[q]	X																			X
QOL assessments[r]		X						X									X			
Dose study drug		Daily for 28 days (4 weeks)									Daily for 28 days 4 weeks)									
Dispense study drug[s]		X									X									
Collect study drug, assess compliance[s]		X			X			X			X			X			X	X		X

Abbreviations: ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; COVID-19, coronavirus disease 2019; DBS, dried blood sample; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; EOS, End of Study; EQ-5D, European Quality of Life - 5 Dimensions; EQ-5D-5L, 5-Level European Quality of Life - 5 Dimensions; EQ-5D-Y, European Quality of Life - 5 Dimensions for Youth; ETV, Early Termination Visit; GGT, gamma glutamyl transferase; HEENT, head, eyes, ears, nose, and throat; LDH, lactate dehydrogenase; Phe, phenylalanine; PKU-QOL, Phenylketonuria-Quality of Life; QOL, quality of life; RBC, red blood cell; SAE, serious adverse event; TP, Treatment Period; Tyr, tyrosine; WBC, white blood cells

[a] The washout period consists of a minimum 14-day washout (may be longer depending on timing for determination of response). During the washout period, study drug will not be administered, and compliance will not be assessed. It is acceptable for Day 14 of the Washout Period 1 and Day -1 of TP2 to be performed on the same day.
[b] An EOS Visit will be performed on/after Day 14 (+3 days) of Washout Period 2.
[c] An ETV will be performed on or between Day 28 and Day 35 for all participants who are determined to be non-responsive to sepiapterin in Part 1, or within 2 days for any participant who discontinues the study prematurely during Part 1 or Part 2 for any other reason. If eGFR calculation is desired, participants <18 years of age should have their height and serum creatinine measured, and participants ≥18 years of age should have their serum creatinine measured. Additionally, a phone follow-up visit will occur 30 (±3) days after the last dose of study drug to assess for SAEs.
[d] Randomization can occur anytime during the Part 1 sepiapterin washout after confirmation of meeting sepiapterin-responsiveness of blood Phe reduction ≥20% and scheduling of Part 2 Treatment Period 1 Day 1 in-clinic visit.
[e] In-clinic visits will occur for all participants on Part 2 Day 1, and Part 2 Day 28 for each treatment period. There is a ±2-day window permitted for the Part 2 Day 28 visit for each treatment period. Participant should continue treatment until the Day 28 (±2 days) Phe sample has been collected.
[f] Includes blood pressure, pulse, respiratory rate, and temperature. Obtain vital signs prior to collection of any laboratory samples (including Phe). If feasible, participants will rest for 5 minutes in a supine position before vital signs are assessed. Vital signs will be collected both predose and 2 hours postdose on Part 2 Day 1 during each treatment period; for all other timepoints, they will be taken at any time during the visit but prior to collection of any laboratory samples (including Phe).
[g] To be completed prior to initial dosing and at additional timepoints where indicated (eg, 2-hour postdose vital signs).
[h] Height should only be measured for participants <18 years of age (at time of visit) at the indicated visits.
[i] If feasible, participants will rest for 5 minutes in a supine position before ECG is performed. Twelve-lead ECGs will be performed at Part 2 Day 28 for each treatment period and EOS, and at the ETV (if appropriate). Electrocardiograms will be performed in triplicate, with each read taken at least 1 minute apart.
[j] Conduct a complete physical examination of general appearance, dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological, and skin parameters.
[k] Includes clinical chemistry panel (albumin, AP, ALT, AST, B12, BUN, calcium, CO2, chloride, serum creatinine, iron, GGT, glucose, LDH, phosphate, potassium, sodium, total bilirubin, direct bilirubin, total cholesterol, total protein, and uric acid), hematology panel (hematocrit, hemoglobin, platelet count, RBC, WBC, and WBC differential), and urinalysis (bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen, and microscopic examination of WBC, RBC, and epithelial cells). Participants should be fasted prior to collection of samples (minimum of 4 hours for participants <6 years and minimum of 8 hours for participants ≥6 years).
[l] eGFR should be calculated (all participants).
[m] Pregnancy tests are required for all women of childbearing potential; urine pregnancy testing to occur on Days 1 and 28 of each treatment period, and urine testing to occur during all other in-clinic visits. Any positive urine pregnancy test should be confirmed by a serum pregnancy test performed at the site's local laboratory.
[n] Record all treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) starting from 30 days prior to Screening until completion of EOS or ETV (if applicable).
[o] Adverse events will be collected from the time of informed consent and/or assent (as applicable) until completion of EOS or ETV (for participants who discontinue prematurely or are considered non-responsive to sepiapterin after Part 1, participants will continue to be followed for 30 days after last dose for SAEs). If a participant elects not to continue into Study PTC923-MD-004-PKU, they should be followed up 30 days after their EOS Visit for collection of AEs.
[p] Blood Phe/Tyr levels via dried blood sample are measured at each timepoint indicated. Day -1 and Day 1 (predose) samples should be taken predose. All other samples can be collected predose or postdose. Samples will be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day at each collection timepoint. Samples obtained at home will be shipped to the study site or returned to the study site at the next clinic visit. Samples to be collected at home if no clinic visit is planned that day. If clinic visit is planned, sample should be collected in clinic where possible. A window ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.
[q] Participants are to maintain a consistent diet (with respect to Phe intake) during the study. At each in-clinic visit, a dietician will monitor each participant's diet to calculate Phe consumption and to have regular contact with participants. The 3-day diet records will be collected biweekly throughout the study.
[r] PKU-QOL (Parent PKU-QOL [6 to 8 years]; Child PKU-QOL [9 to 11 years]; Adolescent PKU-QOL [12 to 17 years]; Adult PKU-QOL [≥18 years]) will be conducted using the tool in one of the following validated languages: English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the

PKU-QOL will not be required for participants whose primary language is not one of the available validated languages. EQ-5D (EQ-5D-Y Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and EQ-5D-5L [≥16 years]) will also be conducted.

[s] If feasible, study drug should be dispensed and returned at the study site. In the event that a participant cannot go into the study site (ie, due to COVID-19 restrictions, inability to make visit), study drug may be shipped directly to the participant's home/study site. In the event that a participant cannot go into the study site, unused study drug can be shipped directly back to the study site per local guidance.

Note: If any unscheduled visits are performed when eGFR calculation is desired, alongside any assessments/procedures considered appropriate by the Investigator (or designee), participants <18 years of age should have their height and serum creatinine measured, and participants ≥18 years of age should have their serum creatinine measured.

Table 5: Phenylalanine/Tyr Sample Collection (Screening Period)

Evaluation	Screening Period (Up to 45 Days)	
	Screening Visit	BH_4 Washout Period
Study Day	Up to 45 Days	7 Days
DBS	X	Day 1, 4, 7

Abbreviations: BH_4, tetrahydrobiopterin; DBS, dried blood sample; Tyr, tyrosine
Note: Analysis of the screening blood Phe level can be performed at the site's local laboratory for determination of eligibility; however, a separate sample for blood Phe/Tyr should still be sent to the central laboratory for the screening timepoint.

Table 6: Phenylalanine/Tyr Sample Collections (Part 1)

	Part 1 - Open-Label Responsiveness Test							
	Sepiapterin Open-Label Treatment 14 Days					Sepiapterin Washout Minimum 14 Days		
Study Day	-1	1	7	10	14	19	24	28
DBS	X	X	X	X	X	X	X	X

Abbreviations: DBS, dried blood sample; ETV, Early Termination Visit; Tyr, tyrosine
Note: If a participant discontinues the study early, an additional DBS should be taken at the ETV.
Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
Day -1 and Day 1 samples for Part 1 should be taken predose. All other samples can be collected predose or postdose.
Note: A window ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.

Table 7: Phenylalanine/Tyr Sample Collections (Part 2) and End of Study

	Sepiapterin 60 mg/kg vs Sapropterin 28 Days (4 Weeks)								Washout 1 Minimum 14 Days			Sepiapterin 60 mg/kg vs Sapropterin 28 Days (4 Weeks)								Washout 2 Minimum 14 Days			End of Study
Study Day	-1	1	7	10	14	19	24	28	5	10	14	-1	1	7	10	14	19	24	28	5	10	14	
DBS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: DBS, dried blood sample; ETV, Early Termination Visit; Tyr, tyrosine
Note: If a participant discontinues the study early, an additional DBS should be taken at the ETV.
Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
Day -1 and Day 1 samples for Part 2 should be taken predose. All other samples can be collected predose or postdose.
Note: A window ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.

APPENDIX 2. PSEUDOCODE FOR STATISTICAL ANALYSIS

1. Primary MMRM analysis

```
proc mixed data=xxx;
   class trt seq period avisit subjid;
   model chg = trt seq period avisit bl trt*avisit / s ddfm=kr;
   random subjid(seq);
   repeated avisit / type=un subject=subjid;
   lsmeans trt*avisit / pdiff cl alpha=0.05;
run;
```

2. Multiple imputation

```
proc mi data=xxx out=xxx seed=xxx nimpute=100
   class trt seq period cPKU;
   fcs reg (bl w1-2 w3-4);
   mnar model(bl w1-2 w3-4 / modelobs =(trt='control'));
   var trt seq period age cPKU phecons bl w1-2 w3-4;
run;
```

Note: 100 imputation sets of the study data will be generated using the following variables to model the blood Phe levels: treatment group, sequence, period, age, classical PKU, the average of daily Phe consumption of the 3-day period at screening, and multiple Phe levels during each treatment period of Part 2 which include baseline Phe level, Phe levels at week 1-2 and at week 3-4.

APPENDIX 3. EQ5D INDEX SCORE DERIVATION

3 versions of the EQ-5D will be used: EQ-5D-Y Proxy Version 1 (3 to 7 years), EQ 5D-Y (8 to 15 years), and EQ-5D-5L (≥16 years) measuring 5 domains of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.

EQ-5D-Y Proxy Version 1 (3 to 7 years) and EQ 5D-Y (8 to 15 years) will be derived using the same value set, and will be derived based on Spain value set:

- SAS_Syntaxis Spanish_eq5dY3l_value_set.sas

For EQ-5D-5L, below are the list of documents to be used to derive the domain scores based on US value set:

- US EQ-5D-5L Value Set.xlsx
- US EQ-5D-5L SAS calculation by 5L Health State Profile.xlsx
- US EQ-5D-5L SAS Value Set Calculation by 5L Dimension.sas

  US EQ-5D-5L SAS Value Set Calculation by 5L Health State Profile.sas

9. REFERENCES

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Signature Page for PTC923-PKU-301 Statistical Analysis Plan (SAP) V1.0 - 31MAR20

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Statistics Approval	[redacted] I approve the document(s) 31-Mar-2025 15:18:24 GMT+0000

Statistics Approval	[redacted] I approve the document(s) 31-Mar-2025 15:27:59 GMT+0000

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