STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER:

PTC923-MD-003-PKU

STUDY TITLE:

A PHASE 3 STUDY OF PTC923 IN SUBJECTS WITH PHENYLKETONURIA

13 APRIL 2023

VERSION 1.0

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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-MD-003-PKU final reporting after database lock. The statistical methods described here are based on the analyses proposed in the Final Protocol issued on 24 Jun 2022, Version 5.0.

1.1. Study Design

This is a Phase 3, double-blind, randomized, multicenter efficacy study of PTC923 versus placebo in subjects with phenylketonuria (PKU).

Approximately 80 subjects with PKU aged ≥2 years who achieve a ≥30% reduction from baseline in blood Phe levels during the responsiveness test (Part 1) will be enrolled in the placebo-controlled treatment period to receive either PTC923 or placebo (Part 2). Approximately 178 subjects are expected to be enrolled in Part 1 of the study to achieve this.

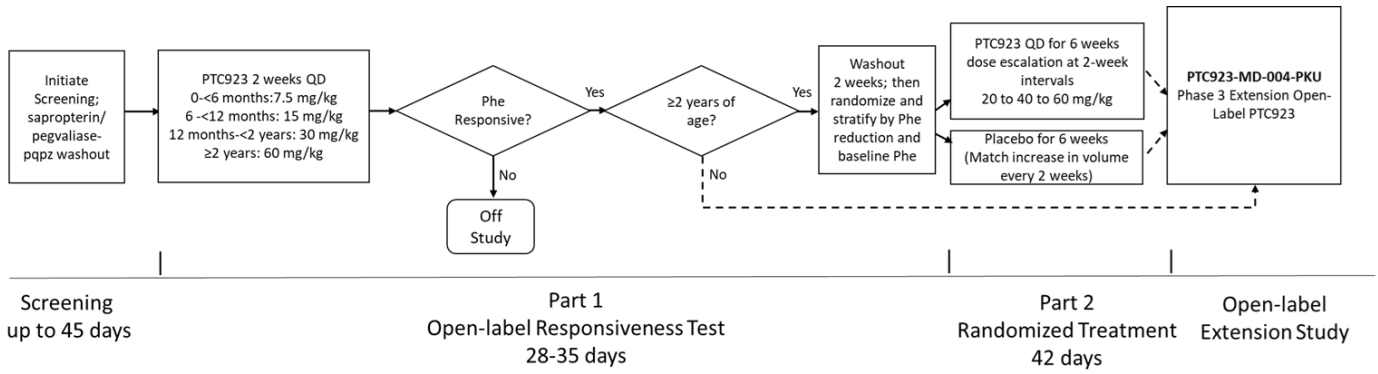
The screening period is up to 45 days. Subjects who enter the study and are receiving pegvaliase-pqpz (PALYNZIQ) supplementation at the Screening Visit must complete a 30-day washout period prior to dosing. Subjects who enter the study and are receiving tetrahydrobiopterin (BH_4) supplementation (eg, sapropterin dihydrochloride, KUVAN) at the Screening Visit must complete a 7-day washout period prior to first dose with PTC923.

Part 1 of the study tests for responsiveness to PTC923, with 14 days of open-label treatment with PTC923 7.5 mg/kg (subjects 0 to <6 months of age), 15 mg/kg (subjects 6 to <12 months of age), 30 mg/kg (subjects 12 months to <2 years of age), or 60 mg/kg (subjects ≥2 years of age) administered orally once a day followed by a minimum of 14 days PTC923 washout. At the end of treatment in Part 1, the mean change in blood phenylalanine (Phe) levels over the 14-day treatment period for all subjects will be assessed against their pretreatment blood Phe level. The pretreatment blood Phe level will be the mean of Day -1 and Day 1 (predose) blood Phe levels. Subjects ≥2 years of age who experience a <15% reduction in blood Phe levels will be classified as nonresponsive and participation in the study will be terminated. Subjects (≥2 years of age) who experience a ≥15% reduction in blood Phe levels will continue into Part 2, which is a randomized, placebo-controlled, double-blind treatment period. Subjects <2 years of age who experience ≥15% reduction in blood Phe levels will also be classified as responsive and offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU. Subjects <2 years of age who experience a <15% reduction in blood Phe levels will be classified as nonresponsive and will not be offered the option to enroll directly into the open-label extension study. Following the minimum 14-day PTC923 washout period, all eligible subjects ≥2 years of age will be randomized in Part 2 to receive either PTC923 or placebo. Subjects will be stratified based on their mean percent reduction in blood Phe levels (ie, subjects with mean % reduction in Phe levels of ≥15% to <30% and subjects with mean % reduction in Phe levels of ≥30%), and their baseline blood Phe concentration (ie, subjects with baseline blood Phe <600 µmol/L and subjects with baseline blood Phe ≥600 µmol/L). Within each stratum, subjects will be randomized to either PTC923 or placebo in 1:1 ratio.

Subjects randomized to PTC923 will receive PTC923 20 mg/kg daily for Weeks 1 and 2 (Days 1 to 14), then PTC923 40 mg/kg daily for Weeks 3 and 4 (Days 15 to 28), then PTC923 60 mg/kg daily for Weeks 5 and 6 (Days 29 to 42). As dosing is weight-based and to maintain the blind, subjects randomized to placebo will receive equivalent quantities of placebo to match

the 20 to 40 to 60 mg/kg dose escalation of the PTC923 treatment arm. After 6 weeks of treatment with either PTC923 or placebo, subjects will be offered the option to enter the open-label PTC923-MD-004-PKU study. Approximately 45 centers will participate in this study. The study design is summarized in Figure 1 and the Schedule of Assessments is presented in Table 2 (Screening Period) and Table 3 (Parts 1 and 2).

Figure 1: Study Schema for Study PTC923-MD-003-PKU



Abbreviations: Phe, phenylalanine
Note: Phe responsiveness is defined as mean Phe reduction ≥15%.

PK Substudy Only:

Approximately 30 subjects ≥12 years of age will participate in the PK substudy. At least 12 out of the 30 subjects ≥12 years of age participating in the PK substudy are expected to be female. In addition, up to 6 subjects from each of these age groups will be included: 1) <2 years; 2) 2 to 5 years; and 3) 6 to 11 years. Blood samples will be collected from subjects participating in PK substudy to characterize the PK of sepiapterin and BH_4 following an intensive schedule on Part 1 Day 1 and Part 1 Day 2 for subjects ≥2 years. For subjects <2 years of age participating in the substudy, blood samples (2 on each occasion; total of 4 samples) will be collected on Part 1 Day 1 and Part 1 Day 14.

In addition to blood drawn for PK sampling at specified timepoints, a small amount of blood will be drawn to a MiniCollect tube (~0.25 mL) at the same time and applied to the VAMS device for blood Phe and Tyr measurement.

For Part 2, blood samples (2 samples/occasion) will be collected from subjects in Part 2 on Days 1, 14, 28, and 42 visits to characterize the PK of sepiapterin and BH_4 at predose (approximately 24 hours post the dose on prior day, except for Day 1, and prior to the dose of the day) and 4 hours postdose.

1.2. Study Objectives

1.2.1. Primary Objective

The primary objective is to evaluate the efficacy of PTC923 in reducing blood Phe levels in subjects with PKU as measured by mean change in blood Phe levels from baseline to Weeks 5 and 6 (ie, the average of each respective treatment dose 2-week period of double-blind treatment).

1.2.2. Secondary Objectives

- To evaluate the proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of the double-blind treatment period
- To evaluate the effect of PTC923 dose response on reducing blood Phe levels in subjects with PKU
- To evaluate the PK of PTC923 in subjects with PKU
- To assess the safety of PTC923 in subjects with PKU

1.2.3. Exploratory Objective

The exploratory objective is to evaluate changes in blood Tyr over time, including the Phe:Tyr ratio.

1.3. Study Endpoints

1.3.1. Primary Endpoint

The primary endpoint of this study is the mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2 double-blind phase. Baseline is defined as the average of Day -1 and Day 1 predose blood Phe levels in Part 2, and mean level at Weeks 5 and 6 is calculated as the average of blood Phe levels collected during the Week 5-6 analysis visit window as described in Section 3.4.4

1.3.2. Secondary Endpoints

Proportion of Subjects with Baseline Phe levels ≥600 µmol/L who Achieve Phe Levels <600 µmol/L at the End of the Double-Blind Treatment Period

For this endpoint, the baseline is defined as the average of Day -1 and Day 1 predose blood Phe levels in Part 2, and Phe level at the end of the double-blind treatment period is referring to the average of blood Phe levels collected during the Week 5-6 analysis visit window as described in Section 3.4.4. The binary endpoint will be calculated for all subjects with baseline Phe levels ≥600 µmol/L: "Achieved" if Phe levels <600 µmol/L at the end of the double-blind treatment period, and "Not Achieved" if Phe levels still ≥600 µmol/L at the end of the double-blind treatment period.

Proportion of Subjects with Baseline Phe levels ≥360 µmol/L who Achieve Phe Levels <360 µmol/L at the End of the Double-Blind Treatment Period

For this endpoint, the baseline is defined as the average of Day -1 and Day 1 predose blood Phe levels in Part 2, and Phe level at the end of the double-blind treatment period is referring to the average of blood Phe levels collected during the Week 5-6 analysis visit window as described in Section 3.4.4. The binary endpoint will be calculated for all subjects with baseline Phe levels ≥360 µmol/L: "Achieved" if Phe levels <360 µmol/L at the end of the double-blind treatment period, and "Not Achieved" if Phe levels still ≥360 µmol/L at the end of the double-blind treatment period.

PTC923 Dose Response on Reducing Blood Phe Levels in Subjects with PKU

The mean change and percent change on blood Phe levels at each dose level from baseline will be calculated to evaluate the dose response of PTC923. Baseline is the average of Day -1 and Day 1 predose blood Phe levels in Part 2. Phe levels at each dose will be calculated by using the analysis visit window as described in Section 3.4.4:

- 20 mg/kg QD: the average of blood Phe levels collected during Weeks 1 and 2
- 40 mg/kg QD: the average of blood Phe levels collected during Weeks 3 and 4
- 60 mg/kg QD: the average of blood Phe levels collected during Weeks 5 and 6

PK of PTC923 in Subjects with PKU

Besides Sepiapterin and BH4 plasma concentrations, pharmacokinetic parameters include C_{max}, T_{max}, $T_{1/2}$, area under the plasma concentration-time curve from time zero to 24 hours (AUC_{0-24h}), area under the plasma concentration-time curve from time zero to infinity (AUC_{0-inf}), CL/F, and Vz/F.

Safety of PTC923 in Subjects with PKU

Safety and tolerability of PTC923 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 over time

Mean change and percent change from baseline in blood Phe:Tyr ratio over time

1.4. Sample Size

All subjects ≥2 years of age with ≥15% response to PTC923 will be eligible to be randomized in Part 2 of the study. Subjects will be stratified in Part 2 based on their mean percent blood Phe reduction in Part 1 (ie, subjects with mean % reduction in blood Phe levels of ≥15% to <30% and subjects with mean % reduction in blood Phe levels of ≥30%). Within each stratum, subjects will be randomized and allocated 1:1 to receive either PTC923 or placebo. Enrollment will continue until approximately 80 subjects from the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1 have been randomized. Additionally, subjects will be stratified based on their baseline blood Phe concentration (ie, subjects with baseline blood Phe <600 µmol/L and subjects with baseline blood Phe ≥600 µmol/L) and evenly distributed to either PTC923 or placebo treatment arms.

For the primary endpoint with a sample size of 80 subjects for the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1, the power to detect a difference in reduction in blood Phe between PTC923 and placebo is >95%, assuming a treatment difference of 250 µmol/L and a within-treatment-group standard deviation of 250 µmol/L, with a 2-sided α of 0.05.

For the primary endpoint for all subjects in Part 2, the power to detect a difference in reduction in blood Phe between PTC923 and placebo is >95%, assuming a treatment difference of 200 μmol/L, a within-treatment-group standard deviation of 250 μmol/L, and a total sample size of 106, with a 2-sided α of 0.05. This sample size assumes approximately 60% of subjects ≥2 years of age enrolled in Part 1 experience a ≥15% reduction in Phe levels.

Approximately 80 subjects with PKU aged ≥2 years of age who achieve a ≥30% reduction from baseline in blood Phe levels during the responsiveness test (Part 1) will be enrolled in the placebo-controlled treatment period to receive either PTC923 or placebo (Part 2). Approximately 178 subjects are expected to be enrolled in Part 1 of the study to achieve this.

Subjects with classical PKU that is biochemically diagnosed (ie, blood Phe birth levels ≥1200 μmol/L and/or historical evidence of Phe concentrations ≥1200 μmol/L in their medical history) will be capped at 20% of the total study population.

1.5. Randomization

In Part 1 of the study, all subjects will receive open-label treatment with PTC923 administered orally once a day. Following the minimum 14-day PTC923 washout period, all eligible subjects will proceed to Part 2 and be randomized to receive either PTC923 or placebo.

Part 2 of this study will be performed in a double-blinded fashion. The investigator and study staff (including processing laboratory personnel), the subjects, and the sponsor's staff will remain blinded to the treatment until study closure. The investigational drug and its matching placebo are indistinguishable and will be packaged in the same way. Subjects will be randomized 1:1 to either PTC923 or placebo using a central randomization process. The randomization will be stratified on two strata using a block randomization technique: one on their mean percent reduction in blood Phe levels in Part 1 (ie, subjects with mean % reduction in Phe levels of ≥15% to <30% and subjects with mean % reduction in Phe levels of ≥30%) and the other one on their baseline blood Phe concentration (ie, subjects with baseline blood Phe <600 μmol/L and subjects with baseline blood Phe ≥600 μmol/L in Part 1), and evenly distributed to either PTC923 or placebo treatment arms.

The process will be established and performed centrally by an experienced contract research organization through an Interactive Response Technology system to maximize the integrity and security of the randomization and ensure appropriate access and convenience-of-use by the investigational sites.

Blinding is important for validity of this clinical study, and the blind may be broken only in the event of a medical emergency.

2. ANALYSIS SETS

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

2.1. Full Analysis Set

Full Analysis Set (FAS): All subjects who are randomized and take at least 1 dose of double-blind study drug in Part 2 will be included in the FAS. Subjects will be analyzed according to their randomized treatment. All efficacy analyses will be based on the FAS.

2.2. Safety Analysis Set

All subjects who receive at least 1 dose of study drug, including during Part 1, will be included in the Safety Analysis Set (the overall safety set). Furthermore, the Part 2 Safety Analyses Set for the randomized part of the study will include all subjects who are randomized and take at least 1 dose of double-blind study drug in Part 2. Subjects will be analyzed according to actual treatment received for safety analysis. 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.

2.3. Per-Protocol Analysis Set

The Per-Protocol (PP) Analysis Set will include all subjects in the FAS who meet the study eligibility requirements and have no major protocol deviations that affect the validity of the efficacy measurements. This is a subset of the FAS. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding. Subjects 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 in Part 2, or a valid blood Phe at Day 42 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 subjects in this population will be finalized prior to unblinding at study completion.

2.4. Pharmacokinetic Analysis Set

All subjects who have at least one measurable plasma concentration of sepiapterin or BH_4 will be included in the Pharmacokinetic Analysis Set.

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 5 and 6 in Part 2 double-blind phase, by a treatment policy strategy. Table 1 lists all the estimands for the study.

Table 1: Study Endpoints and Estimands

Endpoint	Population	Intercurrent Events	Population Level Summary	Analysis
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% confidence interval (CI) between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using a mixed model repeated measures (MMRM) method.	Primary analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method.	Secondary analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria, with no major protocol deviations (one stratum in PP)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria, with no major protocol deviations (PP)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe	PKU subjects ≥2 years of age with a reduction in blood	a. Prohibited medication or diet modification:	Treatment difference and corresponding 95% CI between PTC923 and	Sensitivity analysis for

Endpoint	Population	Intercurrent Events	Population Level Summary	Analysis
levels from baseline to Weeks 5 and 6 in Part 2	Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	Data will be used as collected. b. Early discontinuation: Data will be used as collected.	placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method after multiple imputation (MI) assuming missing at random (MAR).	the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method after MI assuming MAR.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method after control-based imputation assuming missing not at random (MNAR).	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method after control-based imputation assuming MNAR.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using a tipping-point analysis.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation:	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using a tipping-point analysis.	Sensitivity analysis for the primary endpoint

Endpoint	Population	Intercurrent Events	Population Level Summary	Analysis
		Data will be used as collected.		
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication: Data will be used as collected. b. Early discontinuation: Data will be used as collected. c. Prohibited diet modification: Data will be set as missing after two consecutive weeks of change of diet in Part 2.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication: Data will be used as collected. b. Early discontinuation: Data will be used as collected. c. Prohibited diet modification: Data will be set as missing after two consecutive weeks of change of diet in Part 2.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication: Data will be used as collected. b. Early discontinuation: Data will be used as collected. c. Prohibited diet modification: Data will be set as missing after two consecutive weeks of change of diet in Part 2.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM method after MI assuming MAR.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol	a. Prohibited medication: Data will be used as collected. b. Early discontinuation:	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using an MMRM	Sensitivity analysis for the primary endpoint

Endpoint	Population	Intercurrent Events	Population Level Summary	Analysis
and 6 in Part 2	inclusion/exclusion criteria (FAS)	Data will be used as collected. c. Prohibited diet modification: Data will be set as missing after two consecutive weeks of change of diet in Part 2.	method after MI assuming MAR.	
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using a t-test.	Sensitivity analysis for the primary endpoint
Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥15% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Treatment difference and corresponding 95% CI between PTC923 and placebo in the mean change from baseline to Weeks 5 and 6 will be estimated using a t-test.	Sensitivity analysis for the primary endpoint
Proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Odds ratio of PTC923 versus placebo along with the 95% CI will be estimated by using a chi-square test.	Analysis of key secondary endpoint
Proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Odds ratio of PTC923 versus placebo along with the 95% CI will be estimated by using a Cochran-Mantel-Haenszel test stratified by randomization stratification factor (Phe reduction during Part 1: ≥15% to <30% or ≥30%).	Analysis of key secondary endpoint

Endpoint	Population	Intercurrent Events	Population Level Summary	Analysis
Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (one stratum in FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Odds ratio of PTC923 versus placebo along with the 95% CI will be estimated by using a chi-square test.	Analysis of key secondary endpoint
Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of Part 2	PKU subjects ≥2 years of age with a reduction in blood Phe levels of ≥30% in Part 1, as defined by the protocol inclusion/exclusion criteria (FAS)	a. Prohibited medication or diet modification: Data will be used as collected. b. Early discontinuation: Data will be used as collected.	Odds ratio of PTC923 versus placebo along with the 95% CI will be estimated by using a Cochran-Mantel-Haenszel test stratified by randomization stratification factor (Phe reduction during Part 1: ≥15% to <30% or ≥30%).	Analysis of key secondary endpoint

3.2. Interim Analysis

No interim analysis is planned for this study. The DSMB will review accumulating safety data to ensure the safety of study subjects and provide recommendations concerning continuation, termination, or modification of the study. The DSMB chair will review the prespecified data every month (for a total of approximately 15 months), and the DSMB will review the prespecified data every other month and discuss in a bi-monthly scheduled meeting.

3.3. Multicenter Study

It was estimated that subjects would be screened and enrolled across approximately 45 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. By region/country analysis of the primary endpoint may be explored.

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, the baseline is generally defined as the last available measurement prior to the first dose of PTC923 during the open-label run-in phase. For the analysis in Part 2, the baseline is generally defined as the last available measurement prior to the first dose of study drug (PTC923 or placebo) during the double-blind phase.

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 in the range of 120 to 360 µmol/L (Vockley 2014). 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 PTC923. In Part 1, blood Phe levels will be measured on Days -1, 1 (predose), 5, 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 5, 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 PTC923. Phe responsiveness is defined as mean Phe reduction ≥15%.

Part 2, the randomized, placebo-controlled, double-blind 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) 5, 10, 14, 19, 24, 28, 33, 38, and 42 for all subjects. For Part 2 blood Phe level data, the mean blood Phe level of Days 5, 10, and 14 is for the 2-week period of PTC923 20 mg/kg daily, Days 19, 24, and 28 is for the 2-week period of PTC923 40 mg/kg daily, and Days 33, 38, and 42 is for the 2-week period of PTC923 60 mg/kg daily. Baseline is the mean of Day -1 and Day 1 predose blood Phe levels in Part 2. The mean change from baseline and percent change from baseline in Phe levels will be calculated for each of the 2-week treatment periods for each of the subjects.

VAMS clamshells containing 4 sample sticks will be used to collect blood Phe for each timepoint (ie, in quadruplicate). Duplicative sampling for a sample collection timepoint is being used to ensure that at least 1 sample contains sufficient blood volume necessary for an accurate measurement of blood Phe for that collection. If insufficient blood volume was sampled for VAMS, the analyzed blood Phe value will potentially be biased. If the central lab believes that there is insufficient blood volume in all 4 samples, then they will indicate that blood Phe level for that day is insufficient, and the blood Phe value for that study day will be treated the same as not evaluable. 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. As subjects ≥2 years of age who experience a <15% reduction in blood Phe levels (will be classified as nonresponsive) or subjects <2 years of age will not be eligible to proceed to Part 2 randomization, 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 (≥15% to <30% or ≥30%, if participated in Part 2) using frequency tables 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 PTC923 and placebo groups. Subjects who only participated in Part 1 include subjects ≥2 years of age non-responders (subjects ≥2 years of age who experience a <15% reduction in blood Phe levels), subjects <2 years of age regardless of responsiveness to PTC923, early termination subjects during Part 1, and subjects who randomized but did not take any study drug during Part 2.
- For the FAS, the treatment groups can be composed of PTC923 and placebo, 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 (≥15% to <30% or ≥30%) for the subjects who are randomized and treated in Part 2 (FAS).
- Further in Part 2, as subjects will proceed through a dose escalation process, data collected in Part 2 may be presented by the two-week intervals, aligning with the dose levels of 20, 40, or 60 mg/kg QD.

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 randomization date. 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 5	Part 1 Day 5	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 Day -1	Part 2 Day -1	Part 2 Day -1
Part 2 Day 1	Part 2 Day 1	Part 2 Day 1
Part 2 Day 5	Part 2 Day 5	Part 2 Day [2, 7]
Part 2 Day 10	Part 2 Day 10	Part 2 Day [8, 12]
Part 2 Day 14	Part 2 Day 14	Part 2 Day [13, 17]
Part 2 Day 19	Part 2 Day 19	Part 2 Day [18, 22]
Part 2 Day 24	Part 2 Day 24	Part 2 Day [23, 26]
Part 2 Day 28	Part 2 Day 28	Part 2 Day [27, 31]

Analysis Visit	Scheduled Study Day	Analysis Window (in Study Days)
Part 2 Day 33	Part 2 Day 33	Part 2 Day [32, 36]
Part 2 Day 38	Part 2 Day 38	Part 2 Day [37, 39]
Part 2 Day 42	Part 2 Day 42	Part 2 Day [40, 14 days after the last dose in Part 2]
Part 2 Week 1-2		Part 2 Day [2, 17]
Part 2 Week 3-4		Part 2 Day [18, 31]
Part 2 Week 5-6		Part 2 Day [32, 14 days after the last dose in Part 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 5 and 6 in Part 2 double-blind phase will be performed in the stratum with mean percent reduction in blood Phe levels of ≥30% during Part 1, at the significance level of 0.05 (2-sided). If $p<0.05$, the study will be declared successful in this stratum.

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

- Mean change in blood Phe levels from baseline to Weeks 5 and 6 in Part 2 double-blind phase based on the FAS
- Proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of the double-blind treatment period, in the stratum with mean percent reduction in blood Phe levels of ≥30% during Part 1
- Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of the double-blind treatment period, in the stratum with mean percent reduction in blood Phe levels of ≥30% during Part 1
- Proportion of subjects with baseline Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L at the end of the double-blind treatment period, based on the FAS
- Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of the double-blind treatment period, based on the FAS

3.5. Changes to Protocol Specified Analysis

Change	Description
1	An additional secondary endpoint (Proportion of subjects with baseline Phe levels ≥360 µmol/L who achieve Phe levels <360 µmol/L at the end of the double-blind treatment period), 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 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 strata (≥15% 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, and the number of subjects who prematurely discontinue study drug during Part 1 and during Part 2 (along with the reason for the premature termination), completed Part 2 Day 42 visit and continuing in the PTC923 open-label study (Study PTC923-MD-004-PKU) will be summarized.

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 groups. 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 (0 to <6 months, 6 to <12 months, 12 months to <2 years, 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, Australian Aboriginal or Torres Strait Islander, and Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported, Unknown)
- 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 group.

A summary will be provided by treatment groups 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 group.

Classical PKU (ie, blood Phe birth levels ≥1200 µmol/L and or historical evidence of Phe concentrations ≥1200 µmol/L in their medical history) will be summarized by treatment groups.

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 group.

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 group.

Procedure data will be summarized descriptively by treatment group.

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 of the study. Data will be summarized by treatment groups 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

Compliance will be assessed in terms of the percentage of study day that the study drug administrated as prescribed.

$$Compliance\ (\%) = \frac{Actual\ days\ drug\ administered\ as\ prescribed\ during\ the\ treatment\ period}{Expected\ days\ during\ the\ treatment\ period} \times 100$$

This will be calculated by part for each dose level and overall. Data will be summarized descriptively by treatment group.

4.9. Quality of Life Baseline Assessments

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 Day 1 (predose). 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.

European Quality of Life – 5 Dimensions

The European QOL-5 dimensions (EQ-5D) questionnaire will be performed at Part 2 Day 1 (predose). 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.

5. EFFICACY ANALYSIS

5.1. Primary Endpoint Analysis

The primary efficacy endpoint is the mean change in blood Phe levels from baseline to Weeks 5 and 6 (average over a 2-week period) in the Part 2 double-blind phase.

The null hypothesis is that the mean change in blood Phe levels from baseline to Weeks 5 and 6 is the same between PTC923 arm and placebo arm, versus the alternative that they are different, at the 2-sided 0.05 significance level.

A mixed model repeated measures (MMRM) model will be used to analyze the blood Phe level data in the stratum with mean percent reduction in blood Phe levels of ≥30% during Part 1. The response variable is the change from baseline in blood Phe measured at post-baseline assessments for each subject. All blood Phe measurement will be assigned to an analysis visit according to the analysis visit window described in Section 3.4.4. The model will include fixed effects for treatment, baseline blood Phe, baseline Phe level (<600 µmol/L or ≥600 µmol/L), visit (categorical, equivalent to the 2-week dosing periods), and visit-by-treatment interaction. In addition, subject will be included as random effect. 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, confidence interval, and 2-sided p-value will be presented.

If p-value from the above analysis is less than 0.05, the same MMRM model as above will be used on the FAS, with the addition of a fixed effect for blood Phe reduction during Part 1 (≥15% to <30%, or ≥30%).

5.2. Sensitivity Analysis

5.2.1. Analysis on PP Analysis Set

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

5.2.2. Different Methods to Handle Missing Data

For the above primary/secondary analysis, MMRM model implicitly imputes the missing data based on the assumption of missing at random (MAR). In the following sensitivity analyses, the missing data issues will be handled explicitly through multiple imputations (MI) (Rubin 1987, Ouyang 2017). The details on SAS codes are provided in Appendix 2.

- MI under an assumption of MAR
- Control-based imputation: for patients in the PTC923 group who discontinued early, their response distribution after discontinuation would follow the distribution in control group
- Tipping-point analysis: to assess how severe departures from MAR must be in order to overturn the conclusion of the primary analysis

5.2.3. Handling Subjects with Potential Change of Diet

The study requires subjects to continue their usual diet without modification (i.e., no change in daily Phe consumption). To explore the impact of potential change of diet on the treatment effect during Part 2 double-blind phase, a subject's change of Phe consumption (monitored by weekly 3-day diet records), is defined as an increase of more than 100% or a decrease of more than 50% over any two consecutive weeks in Part 2 comparing to screening period. Note the Phe consumption in screening period is calculated by averaging weekly 3-day diet records collected during the entire screening period. If it is determined that more than 5% of subjects are experiencing change of diet, then the blood Phe values collected after the two consecutive weeks will be set as "missing" for those subjects, and the data will be analyzed by the same MMRM model as in primary analysis with and without MI for missing values.

5.2.4. T-test with Completers

A t-test will be used to compare the mean change from baseline in blood Phe measured to Weeks 5 and 6 (average over a 2-week period) in Part 2 between PTC923 and placebo. The analysis will only include subjects who have observations on the endpoint.

5.2.5. Analysis of Phe levels at Steady State

The same MMRM method as in primary analysis will be used to compare the mean change from baseline in blood Phe measured to Weeks 5 and 6, except the average over a 2-week period (including Weeks 1-2, 3-4 and 5-6) will be calculated by using the analysis visit window of the last two days in each 2-week period.

5.3. Subgroup Analysis

The change in blood Phe levels from baseline to Weeks 5 and 6 will be summarized in the following subgroups, in the stratum with mean percent reduction in blood Phe levels of ≥30% during Part 1 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)
- Biochemically diagnosed classical PKU (Yes, No)
- Baseline Phe level of Part 2
  - ≥600 μmol/L, <600 μmol/L
  - ≥360 μmol/L, <360 μmol/L

- Change of diet (Yes, No) during Part 2
  - Follow the definition of "Change of Diet" in Section 5.2.3
- Geographical region (North and Latin America, Europe, Asia Pacific)
- Country

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

- Phe reduction in Part 1 (≥15% to <30%, ≥30%).

5.4. Secondary Endpoints Analysis

Blood Phe levels are collected at the timepoints indicated in Table 4 and Table 5 . The concentration data in Part 1 (collected on Days -1, 1 (predose), 5, 10, and 14 of the treatment period and on Days 15, 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 5, 10, and 14) data will be descriptively summarized by treatment group. The mean change and percent change from the mean baseline will be descriptively summarized too. The following categories (reduction <15%, ≥15% 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 event and percentage by treatment group.

The concentration data in Part 2 (collected on Days -1, 1 (predose), 5, 10, 14, 19, 24, 28, 33, 38, and 42), 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 5, 10 and 14 is for the 2-week period of PTC923 20 mg/kg daily, Day 19, 24 and 28 is for the 2-week period of PTC923 40 mg/kg daily, and Day 33, 38 and 42 is for the 2-week period of PTC923 60 mg/kg daily) will be descriptively summarized by treatment group, along with the mean change and percent change from the mean baseline in Part 2. The following categories (reduction <15%, ≥15% 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 event and percentage by treatment group.

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

The Phe data collected for the PK subgroup subjects will be descriptively summarized by treatment group.

Proportion of Subjects with Baseline Phe Levels ≥600 µmol/L who Achieve Phe Levels <600 µmol/L at the end of the Double-Blind Treatment Period

A binary variable (Achieved versus Not Achieved) will be analyzed by a chi-square test in the stratum with mean percent reduction in Phe levels of ≥30% during Part 1. The odds ratio of PTC923 versus placebo along with the 95% CI will be presented. In order to analyze the data in FAS, a Cochran-Mantel-Haenszel test stratified by randomization stratification factor (Phe reduction during Part 1: ≥15% to <30% or ≥30%) to compare PTC923 versus placebo.

Proportion of Subjects with Baseline Phe Levels ≥360 µmol/L who Achieve Phe Levels <360 µmol/L at the end of the Double-Blind Treatment Period

The same methods as above will be used for the analysis of this endpoint. The odds ratio of PTC923 versus placebo along with the 95% CI will be presented.

PTC923 Dose Response on Reducing Blood Phe Levels in Subjects with PKU

Mean change and percent change from baseline in mean blood Phe levels at each PTC923 dose level (ie, each 2-week period in Part 2) will be assessed using the MMRM model described for the primary endpoint. The LS means, treatment effect estimate, and 95% CI will be presented.

PK Analysis for the PK SubStudy

Descriptive statistics of plasma concentrations of sepiapterin and BH_4 (n, arithmetic mean, SD, coefficient of variation (CV) % of arithmetic mean, median, minimum, maximum, geometric mean, and CV % for the geometric mean) will be presented by age group for both Part 1 and Part 2.

The CV % for the geometric mean will be calculated using the following formula:

$$CV\ (\%) = 100 * \sqrt{e^{SD^2} - 1}$$

where SD is the standard deviation of the natural-log transformed data.

For sepiapterin (PTC923), all concentration values below the lower limit of quantification (LLOQ) will be set to zero. For BH_4, all BLQ concentrations will be set to ½ of LLOQ for predose samples and "missing" for 4 hours postdose samples.

For Part 1, for age ≥2 years old, PK parameter estimates for sepiapterin and BH_4 will be calculated using non-compartmental analysis (NCA) of the plasma concentration versus time data utilizing actual sample collection date and time. The following PK parameters, C_{max}, T_{max}, $T_{1/2}$, $AUC_{0\text{-}24h}$, $AUC_{0\text{-}inf}$, CL/F, and Vz/F for sepiapterin, baseline corrected and uncorrected BH_4 will be descriptively summarized by age groups (2-5, 6-11, ≥12 years old). For <2 years old, PK parameters will not be derived. For Part 2, the observed PK parameters such as Cmax, Tmax will be provided using NCA, if data permits.

PK parameters will be summarized by age group for Part 1, and Part 2 if data permits.

5.5. Exploratory Endpoints

Tyrosine Concentrations

Blood Tyr levels are collected at the same timepoints as blood Phe as indicated in Table 4 and Table 5. VAMS clamshells containing 2 sample sticks will be used to collect blood Tyr for each timepoint (ie, in duplicate). Duplicative sampling for a sample collection timepoint is being used to ensure that at least one sample contains sufficient blood volume necessary for an accurate measurement of blood Tyr for that collection. 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.

The Tyr data collected for the PK subgroup subjects will be descriptively summarized by treatment group.

6. SAFETY ANALYSES

All subjects in the Safety Analysis Set will be included in the analysis by treatment groups. 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 events will be considered:

- Part 1 TEAEs, which includes all AEs occurring after first dose in Part 1 but before first dose in Part 2
- Part 2 TEAEs, which includes all AEs after first randomized dose in Part 2. For Part 2 TEAEs, data will be further presented by each of the dose levels (2-week dosing period) and overall (all 6 weeks) by treatment group.

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, Part 2 Days 1 and 42, and Early Termination Visit (ETV) (if applicable). Vital signs will be collected both predose and 2 hours postdose on Part 1 Day 1 and Part 2 Day 1; for all other timepoints, they will be taken at any time during the visit. Weight will be collected at the Screening Visit, Part 1 Day 1, Part 2 Days 1 and 42, and ETV (if applicable).

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 treatment group, 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 Days 1 and 42, and ETV (if applicable). General appearance; dermatologic; head, eyes, ears, nose, and throat (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 are to be performed at Part 1 Day 1 (predose) and Part 2 Day 42/EOS for all subjects and at the ETV (if applicable). Additional 12-lead ECGs will be performed for all subjects participating in the PK substudy on Part 1 Day 1 at 2, 4, 6, and 8 hours postdose.

The ECG parameters (RR interval, PR interval, QRS interval, QT interval, and QTc interval) and the overall interpretation of ECG (normal, abnormal clinically significant, or abnormal not clinically significant) will be summarized descriptively. Part 1 Day 1 (predose) will be summarized for Part 1 Safety Analysis Set, and along with all the other timepoints data collected for PK substudy will be summarized for the PK Analysis Set. For triplicate measurements, the mean of the three values will be used to summarize.

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 QTc interval prolongation:
  - - QTc interval > 450
  - - QTc interval > 480
  - - QTc interval > 500
- • Change from baseline in QTc interval:
  - - QTc interval increases from baseline >30
  - - QTc 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, B12, and iron
- Clinical chemistry: albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, calcium, CO_2, chloride, serum creatinine, gamma glutamyl transpeptidase, glucose, lactate dehydrogenase, phosphate, potassium, 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 human chorionic gonadotrophin (hCG) (at Screening), urine hCG (at Screening and Part 2 Day 1)

Clinical laboratory test results and laboratory marked abnormalities using pre-defined abnormality criteria will be descriptively summarized for all the specified timepoints by treatment group, 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 1 and Part 2 separately.

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
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 2 and Table 3.

The 3-day diet records will be completed over 3 consecutive days within each period and collected weekly. 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 week 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	13APR2023	Initial release

APPENDIX 1. SCHEDULE OF ASSESSMENTS

Table 3: Schedule of Assessments (Screening Period)

Evaluation	**Screening Period**		
	Screening Visit	**Supplementation Washout Period (Max 30 Days)** [a]	
Study Day	**Up to 45 days**	**Pegvaliase-pqpz 30 days**	**BH_4 7 days**
Informed consent/assent	X		
PAH genotyping[b]	X		
Confirm eligibility	X		
Demographics	X		
Medical history[c]	X		
In-clinic visit	X		
Vital signs, weight, and height[d]	X		
Physical examination[e]	X		
Clinical laboratory tests[f]	X [g]		
Serum/urine pregnancy test [h]	X		
Concomitant medications [i]	Collected throughout the study		
Adverse events [j]	Collected throughout the study		
Blood Phe/Tyr levels [k]	X	Day 1, 20, 30	Day 1, 4, 7
Consistent diet/diet monitoring/3-day diet record distribution/collection [l]	X	X	X

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; BH_4, tetrahydrobiopterin; BUN, blood urea nitrogen; ETV, Early Termination Visit; GGT, gamma glutamyl transferase; HEENT, head, eyes, ears, nose, and throat; LDH, lactate dehydrogenase; PAH, phenylalanine hydroxylase; Phe, phenylalanine; RBC, red blood cell; SAE, serious adverse event; Tyr, tyrosine; WBC, white blood cells

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

[b] To be performed unless documented in subject's medical history.

[c] 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.

[d] 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, subjects will rest for 5 minutes in a supine position before vital signs are assessed. Height will be collected only at Screening.

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

[f] 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, uric acid); hematology panel (hematocrit, hemoglobin, platelet count, RBC, WBC, WBC differential); and urinalysis (bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen and microscopic examination of WBC, RBC, and epithelial cells). Subjects should be fasted prior to collection of samples (minimum of 4 hours for subjects <6 years and minimum of 8 hours for subjects ≥6 years).

[g] eGFR should be calculated for all subjects.

[h] Pregnancy tests required for all women of childbearing potential (Protocol Section 8.1.5); serum testing to occur at the Screening Visit, and urine testing to occur during all other in-clinic visits. Any positive urine pregnancy test should be confirmed by a serum pregnancy test.

[i] Record all treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) starting from 30 days prior to Screening through the study.

[j] AEs will be collected from the time of informed consent and/or assent (as applicable) until completion of Part 2 Day 42 or ETV (for subjects who discontinue prematurely or are considered non-responders after Part 1, subjects will continue to be followed for 30 days after last dose for SAEs). 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 subject is known to be BH_4-responsive). If a subject elects not to continue into Study PTC923-MD-004-PKU, they should be followed up 30 days after their Day 42 visit for collection of AEs.

[k] Blood Phe/Tyr levels via dried blood sample will be measured at each timepoint indicated (Protocol Table 12). Samples will be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day. Blood Phe/Tyr samples can be collected while the subject 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 lab for the screening timepoint. Vital signs should be collected prior to blood Phe/Tyr collection at the Screening Visit.

[l] Subjects are to maintain a consistent diet (with respect to Phe intake) during the study. A dietician will monitor each subject's diet to calculate Phe consumption and to have regular contact with subjects. 3-day diet records will be given to subjects at the screening visit and are to be completed weekly throughout the study. The 3-day diet records will be collected weekly throughout the study.

Table 4: Schedule of Assessments (Part 1 and Part 2 Periods)

Evaluation	**Part 1-Open-Label Responsiveness Test**									**Part 2-Randomized, Placebo-Controlled, Double-Blind Treatment Period**											**ETV** [u]
	PTC923 Open-Label Treatment 0 to <6 months: 7.5 mg/kg 6 to <12 months: 15 mg/kg 12 months to <2 years: 30 mg/kg ≥2 years: 60 mg/kg 14 Days						**PTC923 Washout[a] Minimum of 14 Days**			**PTC923 20>>40>>60 mg/kg vs Placebo) 42 Days (6 Weeks)**											
Study Day	**-1**	**1**	**2**	**5**	**10**	**14**	**19**	**24**	**28[b]**	**-1**	**1**	**5**	**10**	**14**	**19**	**24**	**28**	**33**	**38**	**42/ EOS**	
Confirm eligibility									X												
Enrollment		X																			
Randomization										X[c]											
In-clinic visit		X	X[d]			X[d]					X			X[d]			X[d]			X	X
Virtual visit						X			X[e]					X			X				
At home assessment/ procedure	X																				
Vital signs[f], weight		X[g]									X[g]									X	X
Height [h]																				X [u]	
ECGs[i]		X[g]																		X	X
Physical examination[j]		X[g]									X[g]									X	X
Clinical laboratory tests[k]		X[g]									X[g]									X[l]	X
Serum/urine pregnancy test [m]		X[g]									X[g]									X	X
Concomitant medications[n]	Collected throughout the study																				
Adverse events[o]	Collected throughout the study																				
Blood Phe/Tyr levels (DBS)[p]	Sample to be taken at all timepoints																				
Blood for PK substudy[q]		X	X			X					X			X			X			X	
Consistent diet/diet monitoring /3-day diet record[r]		X									X										X
QOL Assessments[s]											X										

Evaluation	**Part 1-Open-Label Responsiveness Test**									**Part 2-Randomized, Placebo-Controlled, Double-Blind Treatment Period**											**ETV**[u]
	PTC923 Open-Label Treatment 0 to <6 months: 7.5 mg/kg 6 to <12 months: 15 mg/kg 12 months to <2 years: 30 mg/kg ≥2 years: 60 mg/kg 14 Days						**PTC923 Washout[a] Minimum of 14 Days**			**PTC923 20>>40>>60 mg/kg vs Placebo) 42 Days (6 Weeks)**											
Study Day	**-1**	**1**	**2**	**5**	**10**	**14**	**19**	**24**	**28[b]**	**-1**	**1**	**5**	**10**	**14**	**19**	**24**	**28**	**33**	**38**	**42/ EOS**	
Dose study drug		Daily for 14 days									Daily for 42 days (6 weeks)										
Dispense study drug		X									X										
Collect study drug, assess compliance						X^t					X^t			X^t			X^t			X^t	X^t

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; EOS, End of Study; ETV, Early Termination Visit; 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; GGT, gamma glutamyl transferase; HEENT, head, eyes, ears, nose, and throat; LDH, lactate dehydrogenase; Phe, phenylalanine; PK, pharmacokinetics; PKU-QOL, Phenylketonuria-quality of life; QOL, quality of life; RBC, red blood cell; SAE, serious adverse event; Tyr, tyrosine; VAMS, volumetric absorptive microsampling; WBC, white blood cells

[a] PTC923 washout period consists of a minimum 14-day washout (may be longer depending on timing for determination of response). During the PTC923 washout period, study drug will not be administered, and compliance will not be assessed.

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

[c] Randomization can occur anytime during the PTC923 washout after confirmation of meeting PTC923-responsiveness of blood Phe reduction ≥15% and scheduling of Part 2 Day 1 in-clinic visit.

[d] In-clinic visits will occur for all subjects on Part 1 Day 1, Part 2 Day 1, and Part 2 Day 42. Only for subjects participating in the PK substudy, additional in-clinic visits will also occur on Part 1 Day 2, Part 1 Day 14, Part 2 Day 14, and Part 2 Day 28 to obtain PK blood draws. There is a ±2-day window permitted for the Part 2 Day 42 visit. Subject should continue treatment until the Day 42 (±2 days) Phe sample has been collected. For subjects participating in the PK sub-study, if an in-clinic visit is scheduled for PK blood sampling, doses will be administered at the clinic with a meal.

[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). If feasible, subjects 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 1 Day 1 and Part 2 Day 1; for all other timepoints, they will be taken at any time during the visit but prior to collection of any laboratory samples (including Phe). For subjects participating in the PK sub-study, windows for study days where PK samples are taken are presented in Protocol Table 17. For all other timepoints, Protocol Table 2 should be followed.

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

[h] Height should only be measured for subjects <18 years of age (at time of visit) at the indicated visits.

[i] If feasible, subjects will rest for 5 minutes in a supine position before ECG is performed. Twelve-lead ECGs are to be performed at Part 1 Day 1 (predose) for all subjects, Part 2 Day 42/EOS, and at the ETV (if appropriate). Additional 12-lead ECGs will be performed for all subjects participating PK substudy at Part 1 Day 1 at 2, 4, 6, and 8 hours postdose. Electrocardiograms will be performed in triplicate, with each read taken at least 1 minute apart. For subjects participating in the PK substudy, windows for study days where PK samples are taken are presented in Protocol Table 18. For all other timepoints, Protocol Table 2 should be followed.

[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, CO_2, chloride, serum creatinine, iron, GGT, glucose, LDH, phosphate, potassium, sodium, total bilirubin, direct bilirubin, total cholesterol, total protein, uric acid); hematology panel (hematocrit, hemoglobin, platelet count, RBC, WBC, WBC differential); and urinalysis (bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen and microscopic examination of WBC, RBC, and epithelial cells). Subjects should be fasted prior to collection of samples (minimum of 4 hours for subjects <6 years and minimum of 8 hours for subjects ≥6 years).
[l] eGFR should be calculated (all subjects).
[m] Pregnancy tests required for all women of childbearing potential (Protocol Section 8.1.5); serum testing to occur on Part 2 Day 1, and urine testing to occur during all other in-clinic visits (excluding in-clinic visits specifically for the PK substudy). Any positive urine pregnancy test should be confirmed by a serum pregnancy test.
[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 Part 2 Day 42 or ETV (if applicable).
[o] AEs will be collected from the time of informed consent and/or assent (as applicable) until completion of Part 2 Day 42 or ETV (for subjects who discontinue prematurely or are considered nonresponders after Part 1, subjects will continue to be followed for 30 days after last dose for SAEs). If a subject elects not to continue into Study PTC923-MD-004-PKU, they should be followed up 30 days after their Day 42 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 (pre-dose) samples for both Part 1 and Part 2 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. 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] Blood samples will be collected for PK analysis from subjects participating PK substudy at the following timepoints.
In Part 1, subjects ≥2 years, samples will be collected at Part 1 Day 1 (predose, and 0.5, 1, 2, 4, 6, 8, and 24 hours postdose).
In Part 1, subjects <2 years, samples will be collected at Part 1 Day 1 (predose and 4 hours postdose), Part 1 Day 14 (2 and 6 hours postdose).
In Part 2, samples will be collected on Days 1, 14, 28, and 42 at predose (approximately 24 hours post the dose on prior day, except for Day 1) and 4 hours postdose.
In Part 1, for subjects participating in the PK substudy, additional small amount (~0.25 mL) of blood will be collected at the same time of PK sample collection and applied to VAMS for blood Phe and Tyr measurement.
[r] Subjects are to maintain a consistent diet (with respect to Phe intake) during the study. At each in-clinic visit, a dietician will monitor each subject's diet to calculate Phe consumption and to have regular contact with subjects. The 3-day diet records will be collected weekly throughout the study.
[s] Phenylketonuria quality of life (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 (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. 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.
[t] If feasible, study drug should be dispensed and returned at the study site. In the event that a subject cannot go into the study site (ie, due to COVID-19 restrictions, inability to make visit), study drug may be shipped directly to the subject's home/study site. In the event that a subject cannot go into the study site, unused study drug can be shipped directly back to the study site per local guidance.
[u] An ETV will be performed on or between Day 28 to Day 35 for all subjects who are determined to be non-responders in Part 1, or within 2 days for any subject who discontinues the study prematurely during Part 1 or Part 2 for any other reason. If eGFR calculation is desired, subjects <18 years of age should have their height and serum creatinine measured and subjects ≥18years 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.
Note: If any unscheduled visits are performed when eGFR calculation is desired, alongside any assessments/procedures considered appropriate by the Investigator (or designee), subjects <18 years of age should have their height and serum creatinine measured and subjects ≥18years of age should have their serum creatinine measured.

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

Evaluation	Screening Period (up to 45 days)		
	Screening Visit	Supplement Washout Period (Max 30 Days)	
Study Day	Up to 45 Days	Pegvaliase-pqpz 30 Days	BH_4 7 Days
DBS	X	Day 1, 20, 30	Day 1, 4, 7

Abbreviations: BH_4, tetrahydrobiopterin; DBS, dried blood sample; Tyr, tyrosine
[a] 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 lab for the screening timepoint.

Table 6: Phenylalanine/Tyr Sample Collections (Part 1 and Part 2) [a]

Evaluation	Part 1 - Open-Label Responsiveness Test									Part 2 - Randomized, Placebo-Controlled, Double-Blind Treatment Period										
	PTC923 Open-Label Treatment 14 Days						PTC923 Washout Minimum 14 Days			PTC923 20>>40>>60 mg/kg vs Placebo) 42 Days (6 Weeks)										
Study Day	-1	1	2[b]	5	10	14	19	24	28	-1	1	5	10	14	19	24	28	33	38	42
DBS	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 subject 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 both Part 1 and Part 2 should be taken pre-dose. All other samples can be collected pre-dose or post-dose.
[a] 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.
[b] For subjects participating in the PK substudy only.

Table 7: Vital Signs Sampling Times (±Permitted Windows) for Subjects in PK Substudy on PK Sampling Days

Scheduled Sample Time	Permitted Time Window
Predose	Within 1 hour prior to dose
Postdose	±15 minutes

Note: This table relates to study days where PK samples are taken. For all other timepoints, vital signs measurements should be taken as outlined in Table 2.

Table 8: Electrocardiogram Sampling Times (±Permitted Windows)

Scheduled Sample Time	Permitted Time Window
Predose	Within 1 hour prior to dose
≤4 hours after study drug administration	±15 minutes
>4 hours and ≤24 hours after study drug administration	±30 minutes

Note: This table relates to study days where PK samples are taken. For all other timepoints, ECGs should be performed as outlined in Table 2.

Table 9: Pharmacokinetic Sampling Times (±Permitted Windows) for Plasma Samples for Part 1

		Day 1 (Hours)*								Day 14 (Hours)			
		Predose	Postdose							Predose	Postdose		
			0.5	1	2	4	6	8	24		2	4	6
Age (Years)	Window	≤60 Min of Dose	±5 Min			±10 Min		±30 Min		≤60 Min	±5 Min	±10 Min	
<2		X				X					X		X
≥2		X	X	X	X	X	X	X	X				

Note: Additionally, a small amount (~ 0.25 mL) blood will be collected at the same time of PK sample collection and applied to VAMS for blood Phe/Tyr measurement.

Table 10: Pharmacokinetic Sampling Times (±Permitted Windows) for Plasma Samples for Part 2

	Day 1, 14, 28, 42	
	Predose	Postdose (4 hours)
Window	Within 60 min prior to dose of the day and approximately 24 hours post dose of the prior day (except for Day 1)	±15 min

APPENDIX 2. DETAILS ON SENSITIVITY ANALYSIS ON MISSING DATA – MULTIPLE IMPUTATION

First, the multiple imputation will be generated using SAS PROC MI under missing at random (MAR). 100 imputations sets of the study data will be generated using the following variables to model the blood Phe values: treatment group, gender, age, classical PKU, region, the average of daily Phe consumption of the 3-day period at screening, and multiple Phe levels in Part 2 which include mean baseline Phe level, mean 2-week treatment Phe level at 20 mg/kg daily, mean 2-week treatment Phe level at 40 mg/kg daily, Part 2 mean 2-week treatment Phe level at 60 mg/kg daily.

```
PROC MI DATA=xxx OUT=xxx SEED=1275 NIMPUTE=100
    ROUND = . . . . . .10 .10 .10 .10 .10 .10 ;
    CLASS TRTPG1N REGION1N SEX cPKU;
    FCS REG (P2BASE P2W1W2 P2W3W4 P2W5W6);
    VAR TRTPG1N REGION1N SEX AGE cPKU PHECON P2BASE P2W1W2 P2W3W4 P2W5W6;
RUN;
```

Each of the simulated data will be analyzed as described in Section 3.1 for the primary endpoint. Then the results will be combined by SAS PROC MIANALYZE to produce the final analysis result. This result should be consistent with MMRM model result, indicating the robustness of the results from primary analysis.

Furthermore, multiple imputation under an assumption of missing not at random (MNAR) will be examined. In these analyses, the multiple imputation approach will be expanded to allow for varying impact of missing data by:

1) imputation completely based on the control group (ie, control-based imputation), for patients who discontinued the study drug.

```
PROC MI DATA=xxx OUT=xxx SEED=1275 NIMPUTE=100
    ROUND = . . . . . .10 .10 .10 .10 .10 .10 ;
    CLASS TRTPG1N REGION1N SEX cPKU;
    FCS REG (P2BASE P2W1W2 P2W3W4 P2W5W6);
    mnar model(P2BASE P2W1W2 P2W3W4 P2W5W6 / modelobs= (TRTPG1N='Placebo'));
    VAR TRTPG1N REGION1N SEX AGE cPKU PHECON P2BASE P2W1W2 P2W3W4 P2W5W6;
RUN;
```

2) incorporating a shift parameter in the imputation model. The range of values for the shift parameter will be explored to assess how severe departures from MAR must be in order to overturn the conclusion from the primary analysis.

```
PROC MI DATA=&data OUT=impute&sj SEED=1275 NIMPUTE=100
    ROUND = . . . . . .10 .10 .10 .10 .10 .10 ;
    VAR TRTPG1N REGION1N SEX AGE cPKU PHECON P2BASE P2W1W2 P2W3W4 P2W5W6;
    CLASS TRTPG1N REGION1N SEX cPKU;
    FCS REG (P2BASE P2W1W2 P2W3W4 P2W5W6);
    mnar adjust( P2BASE /shift=# adjustobs=(TRTPG1N='PTC923'));
    mnar adjust( P2W1W2 /shift=# adjustobs=(TRTPG1N='PTC923'));
    mnar adjust( P2W3W4 /shift=# adjustobs=(TRTPG1N='PTC923'));
    mnar adjust( P2W5W6 /shift=# adjustobs=(TRTPG1N='PTC923'));
```

```
RUN;
```

is the shift parameter, which can range from min value to max value by increment; min, max and increment values will be determined based on the tipping point that reverses the conclusion from the primary analysis.

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

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