CLINICAL PROTOCOL

A PHASE 3 STUDY OF PTC923 IN SUBJECTS WITH PHENYLKETONURIA

PTC923-MD-003-PKU

24 JUNE 2022

VERSION 5.0

PTC THERAPEUTICS
100 CORPORATE COURT
SOUTH PLAINFIELD, NJ 07080 USA

PROTOCOL IDENTIFIERS

Project Code	PTC923
Therapeutic Area	Metabolic Disorders
PTC Therapeutics Substance Identifier	PTC923
IND Number	143698
EudraCT Number	2021-000474-29
Protocol Number	PTC923-MD-003-PKU
Protocol Version	5.0
Protocol Version Date	24 June 2022
Protocol Phase	Phase 3
Protocol Title	A Phase 3 Study of PTC923 in Subjects with Phenylketonuria

PTC THERAPEUTICS PROTOCOL APPROVAL SIGNATURES

Sponsor's Approval

The protocol has been approved by PTC Therapeutics. Reference to Sponsor as "PTC Therapeutics", including PTC Therapeutics, Inc and PTC Therapeutics MP, Inc, collectively includes relevant entities, subsidiaries, and affiliates thereof.

[redacted]
PTC Therapeutics, Inc.

Date

[redacted]
PTC Therapeutics, Inc.

Date

[redacted]
PTC Therapeutics, Inc.

Date

The eSignature page is located on the last page.

PRINCIPAL INVESTIGATOR AGREEMENT AND SIGNATURE

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

Principal Investigator	**Date**

Institution: _____

Address: _____

City: _____

State/Province: _____

Country: _____

Phone: _____

Fax: _____

E-mail: _____

SYNOPSIS

Name of Sponsor/Company: PTC Therapeutics		
Name of Investigational Product: PTC923		
Protocol Number: PTC923-MD-003-PKU	**Phase:** 3	**Country:** Global
Title of Study: A Phase 3 Study of PTC923 in Subjects with Phenylketonuria		
Study Period: 1.75 years		

Objectives:

Primary:

- To evaluate the efficacy of PTC923 in reducing blood phenylalanine (Phe) levels in subjects with phenylketonuria (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)

Secondary:

- 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 pharmacokinetics (PK) of PTC923 in subjects with PKU
- To assess the safety of PTC923 in subjects with PKU

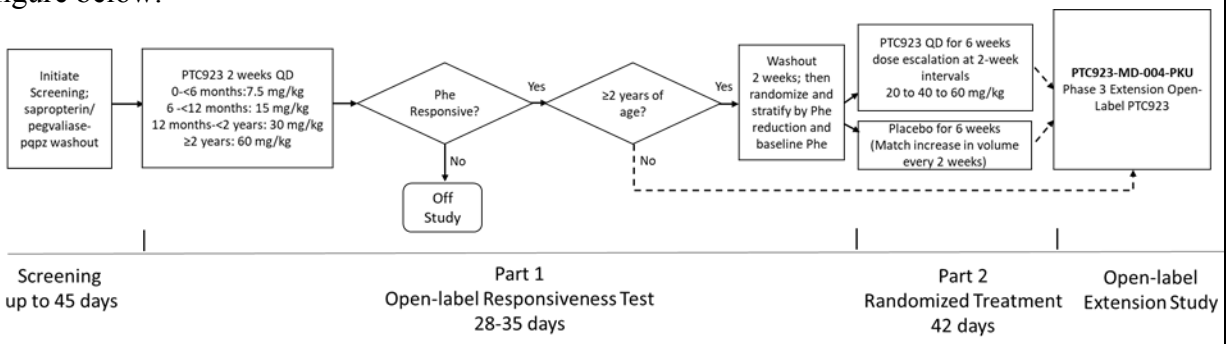
Exploratory Objectives:

- To evaluate changes in blood tyrosine (Tyr) overtime, including the Phe:Tyr ratio

Methodology:

This is a Phase 3, double-blind, randomized, multi-center efficacy study of PTC923 (a synthetic form of sepiapterin) versus placebo in subjects with PKU. 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 14-day PTC923 washout. At the end of treatment in Part 1, the mean change in blood Phe levels over the 14-day treatment period for all subjects will be assessed against their pretreatment blood Phe level. 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 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 be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU. Following the minimum 14-day PTC923 washout period, all eligible subjects ≥2 years of age will be randomized 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 evenly distributed to either PTC923 or placebo treatment arms. 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.
Subjects ≥2 years of age randomized to PTC923 will receive PTC923 20 mg/kg daily for Weeks 1 and 2, then PTC923 40 mg/kg daily for Weeks 3 and 4, then PTC923 60 mg/kg daily for Weeks 5 and 6.
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 42 days of treatment with either PTC923 or placebo, subjects will be offered the option to enter an open-label extension Study PTC923-MD-004-PKU.
Approximately 45 centers will participate in this study. The study design is summarized in the figure below.



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

Screening Period (up to 45 Days):

An informed consent and/or assent (as applicable) form must be signed before any study-related procedures are performed. After providing consent and/or assent (as applicable), subjects will undergo in-clinic screening procedures to determine study eligibility. Subject demographics and medical history information will be collected. Additionally, 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 tetrahydrobiopterin [BH_4]-responsive). Vital signs, weight, and height measurements will be performed. Phenylalanine hydroxylase gene (*PAH*) genotyping should be performed unless already documented in the subject's medical history. Blood Phe/Tyr levels will be measured. A full physical examination will be performed, and blood samples will be collected for clinical laboratory tests and pregnancy testing. Concomitant medication and adverse events (AEs) will be collected from provision of consent and/or assent (as applicable). Subjects will be instructed to continue their usual diet without modification (ie, no change in daily Phe consumption) throughout the entire study. Subjects will maintain a 3-day diet record during each week of Screening, and a dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, have regular contact with subjects, and reinforce the need for subjects to maintain their usual diet without modification. 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 BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) at the Screening Visit must complete a 7-day washout period prior to dosing.

Pegvaliase-pqpz Washout (30 Days):
Following the initial Screening Visit, eligible subjects who are taking pegvaliase-pqpz must discontinue the medication as concomitant treatment/supplementation with pegvaliase-pqpz will not be permitted during the study. Subjects will continue their usual diet without modification (ie, no change in daily Phe consumption) and will maintain a 3-day diet record each week during the Pegvaliase-pqpz Washout. A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification. Blood Phe levels will be measured at each timepoint for the 30-day washout (Days 1, 20, and 30).

BH_4 Supplementation Washout (7 Days):
Following the initial Screening Visit, eligible subjects who are taking BH_4 supplementation must discontinue the medication as concomitant treatment/supplementation with BH_4 will not be permitted during the study. Subjects will continue their usual diet without modification (ie, no change in daily Phe consumption) and will maintain a 3-day diet record during the BH_4 Supplementation Washout. A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification. Blood will be collected for determination of blood Phe levels using dried blood sampling levels at each timepoint for the 7-day washout (Days 1, 4, and 7).

Part 1, Open-Label Responsiveness Test (28 to 35 Days):
Following the initial screening and BH_4 Supplementation/Pegvaliase-pqpz Washout (as necessary), all eligible subjects will be enrolled into Part 1 of the study to test for responsiveness to PTC923. Subjects will receive 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) for 14 days. On Part 1 Day 1, subjects will take their dose of PTC923 while in the clinic; for all other dosing days, subjects will take their dose as an outpatient and prior to coming to the clinic when visits are scheduled. All visits will be outpatient visits or virtual visits. Study visits will occur on Part 1 Day 1, Part 1 Day 2 (PK substudy only), and Part 1 Day 14 (virtual visit, unless in PK substudy).
Blood Phe levels will be measured on Days -1, 1 (predose), 2 (PK substudy only), 5, 10, and 14 of Part 1. Baseline blood Phe level will be calculated as the mean of the blood Phe levels from dried blood samples taken on Day -1 and Day 1 (predose). Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
Subjects will continue their usual diet without modification and will maintain 3-day diet records (ie, one corresponding to each week of the PTC923 open-label treatment period).
A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification.
Subjects participating in the PK substudy: 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 will 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.

Part 1, PTC923 Washout (14 Days Minimum)

Following completion of 14 days of PTC923 open-label treatment, subjects will begin a minimum 14-day washout. No in-clinic study visits are required during the PTC923 washout. In Part 1, blood Phe levels will be measured on Days 19, 24, and 28. Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint. Subjects will continue their usual diet without modification and will maintain a 3-day diet record (ie, one corresponding to each week of the PTC923 washout). A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification.
During the PTC923 washout, the mean change in blood Phe levels over the 14 days of PTC923 treatment (mean of Part 1 Days 5, 10, and 14) will be measured and compared against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 [predose]).
Subjects who experience <15% reduction in blood Phe levels will be classified as nonresponsive and will be contacted to schedule an Early Termination Visit (ETV) (on or between Day 28 to Day 35).
Subjects ≥2 years of age who experience a ≥15% reduction in blood Phe levels will be contacted to confirm their continued eligibility and to schedule the Part 2 Day 1 visit.
Subjects <2 years of age who experience a ≥15% reduction in blood Phe levels will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.
Part 1 for all subjects ends on Day 35. In no circumstances will a subject be permitted to continue in Part 1 beyond Day 35. If a subject's blood Phe concentrations are not available for determination of responsiveness to PTC923 by Day 35 of Part 1, then that subject will be classified as nonresponsive and will be contacted to schedule an ETV and not be eligible to continue into Part 2 of the study or further participation in an open-label extension Study PTC923-MD-004-PKU.

Part 2, Randomized, Double-Blind Treatment Period (42 Days):

All eligible subjects ≥2 years of age will be randomized to receive either PTC923 or placebo for 42 days (ie, 6 weeks) in Part 2, the randomized, placebo-controlled, double-blind treatment period. Subjects will be stratified based on their mean percent blood Phe reduction in Part 1 (mean % reduction in blood Phe levels of ≥15% to <30% and mean % reduction in blood Phe levels of ≥30%) and evenly distributed across both treatment arms (PTC923 or placebo). 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. This portion of the study is double-blinded. Subjects will continue their usual diet without modification and will maintain 3-day diet records (one corresponding to each week of Part 2). A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification. Subjects randomized to PTC923 will receive PTC923 20 mg/kg daily for Days 1 to 14 (ie, Weeks 1 and 2), then PTC923 40 mg/kg daily for Days 15 to 28 (ie, Weeks 3 and 4),

then PTC923 60 mg/kg daily for Days 29 to 42 (ie, Weeks 5 and 6). As dosing is weight-based and to maintain the blind, subjects randomized to placebo will received equivalent quantities of placebo to match the 20 to 40 to 60 mg/kg dose escalation with PTC923.
On Part 2 Day 1, subjects will take their dose of study drug (PTC923 20 mg/kg or placebo) in clinic; for all other dosing days, subjects will take their dose at home and prior to coming to the clinic when visits are scheduled. All visits will be outpatient visits or virtual visits.
Study visits will occur on Part 2 Day 1, Part 2 Day 14 (in-clinic visit for PK substudy only), Part 2 Day 28 (in-clinic visit for PK substudy population), and Part 2 Day 42 to conduct study evaluations, collect 3-day diet record, and capture any AEs and concomitant medications.
Blood Phe levels will be measured in Part 2 on Days -1, 1 (predose), 5, 10, 14, 19, 24, 28, 33, 38, and 42. Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
On Part 2 Day 1 (predose), the Phenylketonuria-quality of life (PKU-QOL) (Parent PKU-QOL [6 to 8 years]; Child PKU-QOL [9 to 11 years]; Adolescent PKU-QOL [12 to 17 years]; Adult PKU-QOL [≥18 years]) will be conducted using the tool in one of the following validated languages: English (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. On Part 2 Day 1 (predose), the European Quality of Life - 5 Dimensions (EQ-5D) (European Quality of Life-5 Dimensions for Youth [EQ-5D-Y] Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and 5-Level European Quality of Life - 5 Dimensions (EQ-5D-5L) [≥16 years]) will be conducted.
PK substudy only: 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.
End of Study:
For subjects <2 years of age, End of Study is defined as their final visit in Part 1 and should occur on/between Part 1 Day 28 and Part 1 Day 35. All subjects <2 years of age who experience a ≥15% reduction in blood Phe levels during Part 1 will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.
For subjects ≥2 years of age who complete Part 2, End of Study is defined as their final study visit on Part 2 Day 42. All subjects who complete Part 2 will be given the opportunity to continue treatment in the PTC923 Open-Label Extension Study, PTC923-MD-004-PKU.
Early Termination Visit:
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. During the ETV, 3-day diet records, AEs, concomitant medications, weight, vital signs, ECGs, and blood Phe/Tyr levels will be collected. A physical examination, clinical laboratory tests, and a pregnancy test will be performed. For non-responsive subjects and subjects who discontinue prematurely, following the End of Study visit, subjects may revert to their prestudy treatment for PKU (at the investigators and the subject's treating physician discretion), and a phone follow-up visit will occur 30 (±3) days after the last dose of study drug to assess for serious adverse events (SAEs).

Sample Size Justification:
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 the subjects ≥2 years of age enrolled in Part 1 experience a ≥15% reduction in Phe levels.

Number of Subjects (Planned):
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.

Diagnosis and Main Criteria for Inclusion:
Subjects with any *PAH* mutation are permitted to screen and enroll into the study. However, subjects with biochemically diagnosed 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 capped at 20% of the total study population. Genotyping will not be required for study eligibility; however, all subjects will undergo genotyping unless documented in their medical history and these data will be collected for analysis.

Inclusion Criteria:

1. Informed consent and assent (if necessary, at the investigator's discretion [ie, for children and/or subjects who are mentally impaired secondary to disease]) with parental/legal guardian consent
2. Male or female subjects of any age
3. Uncontrolled blood Phe level ≥360 µmol/L on current therapy anytime during Screening and uncontrolled blood Phe level ≥360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the subject's medical history (inclusive of the Screening value)
4. Clinical diagnosis of PKU with hyperphenylalaninemia (HPA) documented by past medical history of at least 2 blood Phe measurements ≥600 µmol/L

5. Women of childbearing potential, as defined in (CTFG 2020), must have a negative pregnancy test at Screening and agree to abstinence or the use of at least one highly effective form of contraception (with a failure rate of <1% per year when used consistently and correctly):
   - Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
     - Oral
     - Intravaginal
     - Transdermal
   - Progestogen-only hormonal contraception associated with inhibition of ovulation:
     - Oral
     - Injectable
     - Implantable
   - Intrauterine device
   - Intrauterine hormone-releasing system
   - Bilateral tubal occlusion
   - Vasectomized partner with confirmed azoospermia

   Highly effective contraception or abstinence must be continued for the duration of the study and for up to 90 days after the last dose of the study drug.
   All females will be considered of childbearing potential unless they are postmenopausal (at least 12 months consecutive amenorrhea in the appropriate age group without other known or suspected cause) or have been permanently sterilized surgically (eg, hysterectomy, bilateral salpingectomy, bilateral oophorectomy).
6. Males who are sexually active with women of childbearing potential who have not had a vasectomy must agree to use a barrier method of birth control during the study and for up to 90 days after the last dose of study drug. Males must also refrain from sperm donations during this time period.
   Males who are abstinent will not be required to use a contraceptive method unless they become sexually active. Males who have undergone a vasectomy are not required to use a contraceptive method if at least 16 weeks post procedure.
7. Willing and able to comply with the protocol and study procedures
8. Willing to continue current diet unchanged while participating in the study

Exclusion Criteria:

1. The individual, in the opinion of the investigator, is unwilling or unable to adhere to the requirements of the study
2. Gastrointestinal disease (such as irritable bowel syndrome, inflammatory bowel disease, chronic gastritis, and peptic ulcer disease, etc.) that could affect the absorption of study drug
3. History of gastric surgery, including Roux-en-Y gastric bypass surgery or an antrectomy with vagotomy, or gastrectomy
4. Inability to tolerate oral medication
5. History of allergies or adverse reactions to synthetic BH_4 or sepiapterin
6. Current participation in any other investigational drug study or use of any investigational agent within 30 days prior to Screening

7. Any clinically significant laboratory abnormality as determined by the investigator. In general, each laboratory value from Screening and baseline chemistry and hematology panels should fall within the limits of the normal laboratory reference range, unless deemed not clinically significant by the investigator
8. A female who is pregnant or breastfeeding, or considering pregnancy
9. Serious neuropsychiatric illness (eg, major depression) not currently under medical control, that in the opinion of the investigator or sponsor, would interfere with the subject's ability to participate in the study or increase the risk of participation for that subject
10. Past medical history and/or evidence of renal impairment and/or condition including moderate/severe renal insufficiency (glomerular filtration rate [GFR] <60 mL/min) and/or under care of a nephrologist
11. Any abnormal physical examination and/or laboratory findings indicative of signs or symptoms of renal disease, including calculated GFR <60 mL/min/1.73m^2.
    In subjects ≥18 years of age, the Modification of Diet in Renal Disease Equation should be used to determine GFR.
    In subjects <18 years, the Bedside Schwartz Equation should be used to determine GFR.
12. Requirement for concomitant treatment with any drug known to inhibit folate synthesis (eg, methotrexate)
13. Confirmed diagnosis of a primary BH_4 deficiency as evidenced by biallelic pathogenic mutations in *6-pyruvoyltetrahydropterin synthase*, recessive *GTP cyclohydrolase I*, *sepiapterin reductase*, *quinoid dihydropteridine reductase*, or *pterin-4-alpha-carbinolamine dehydratase* genes
14. Major surgery within the prior 90 days of screening
15. Concomitant treatment with BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) or pegvaliase-pqpz (PALYNZIQ)
16. Unwillingness to washout from BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) or pegvaliase-pqpz (PALYNZIQ)

Investigational Product, Dosage, and Mode of Administration:

The test product is PTC923 Powder for Oral Use. All doses will be administered orally, once a day with food.

Part 1: All subjects will receive PTC923 for 14 days:

- Subjects 0 to <6 months of age: 7.5 mg/kg
- Subjects 6 to <12 months of age: 15 mg/kg
- Subjects 12 months to <2 years of age: 30 mg/kg
- Subjects ≥2 years of age: 60 mg/kg

Part 2: Subjects ≥2 years of age randomized to PTC923 treatment will receive PTC923 (6 weeks in total):

- 20 mg/kg for 14 days
- 40 mg/kg for 14 days
- 60 mg/kg for 14 days

Duration of Treatment:
All enrolled subjects will receive PTC923 for 2 weeks in Part 1. Subjects ≥2 years of age with ≥15% response to PTC923 will continue treatment in Part 2 with PTC923 or placebo for 6 weeks. Subjects <2 years of age with ≥15% response to PTC923 in Part 1 will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

Reference Therapy, Dosage, and Mode of Administration:
The reference therapy is Placebo for PTC923 Powder for Oral Use. In Part 2, subjects ≥2 years of age randomized to placebo will receive Placebo for PTC923 Powder for Oral Use for 6 weeks. All doses will be administered orally, once a day with food.

Criteria for Evaluation:
Efficacy endpoints:
The primary efficacy measure will be reduction in blood Phe levels in subjects with PKU as measured by mean change in Phe levels from baseline to Part 2 Weeks 5 and 6 (ie, the average of the 2-week period at the target dose of double-blind treatment). Baseline blood Phe level will be the mean of Day -1 and Day 1 (predose) blood Phe levels.
The secondary efficacy endpoints are 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 and the change from baseline in mean blood Phe levels at each PTC923 dose level (ie, each 2-week period in Part 2).
The exploratory efficacy endpoint is the change from baseline in blood Tyr over time, including the Phe:Tyr ratio.
Safety endpoints:
Safety and tolerability of PTC923 as measured by severity and number of treatment-emergent adverse events (TEAEs), clinical laboratory tests, vital signs, physical examinations, and electrocardiograms (ECGs) will be compared to placebo.
Pharmacokinetics endpoints:
Depending on the number of samples obtained, the following PK parameters of sepiapterin and BH_4 for a given subject may be calculated using noncompartmental methods: C_{max}, T_{max}, $T_{1/2}$, area under the plasma concentration-time curve from time zero to 24 hours ($AUC_{0\text{-}24h}$), area under the plasma concentration-time curve from time zero to infinity ($AUC_{0\text{-}inf}$), apparent total clearance of the drug from plasma following oral administration (CL/F), and apparent volume of distribution during terminal phase after non-intravenous administration (Vz/F). Additionally, depending on the number of samples collected and subject demography, a population PK analysis will be performed using all PK samples collected in the study to further characterize the PK of sepiapterin and BH_4 to examine potential source of PK variability, such as age, and to explore the relationship between exposure and response.

Statistical Methods:
Analysis Sets:
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.
Per Protocol (PP) Analysis Set: The 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. The PP Analysis Set will be used for sensitivity analysis of the primary efficacy endpoint. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the statistical analysis plan (SAP).

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. Subjects will be analyzed according to actual treatment received.
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.

Efficacy:

All efficacy analyses will be performed based on the FAS. The primary endpoint analysis will also be conducted using the PP Analysis Set.
The null hypothesis of this study is that the mean change in Phe levels from baseline to end of the double-blind treatment period is the same in the PTC923 arm and in the placebo arm versus the alternative that they are different; a 2-sided test at the 5% alpha level will be applied.
For the primary efficacy endpoint, a gatekeeping procedure will be used to control the familywise error rate:
The stratum of subjects ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 will be tested at the significance level of 0.05 (2-sided). If $p<0.05$ then the study will be declared positive in this stratum.
Only if the stratum of subjects ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 is statistically significant at the 0.05 level, will the overall study with all subjects ≥2 years of age with a mean percent reduction in Phe levels of ≥15% during Part 1 be tested, also at the 0.05 significance level.
For the primary endpoint of reduction in blood Phe for the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1, a mixed model repeated measures (MMRM) model will be fitted using the available blood Phe data. The response variable is the change from baseline in blood Phe measured at post-baseline assessments for each subject. The model will include fixed effects for treatment, baseline blood Phe, baseline Phe stratum (<600 μmol/L or ≥600 μmol/L), visit (categorical), 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 for the average change at Part 2 Weeks 5 and 6 will be used to perform treatment group comparisons. The LS means, treatment effect estimate, confidence interval, and 2-sided p value will be presented. Corresponding LS means from the same MMRM model will be used to estimate the treatment effects at each visit week.
For the primary endpoint of reduction in blood Phe for all subjects, the same MMRM model used for the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1 will be used with the addition of a fixed effect for Phe reduction randomization strata (≥15% or ≥30%).

Missing data:
For the primary analysis of the primary endpoint, MMRM model will be used on the available data assuming the missing assessments are missing at random (MAR). Missing data will be imputed using the multiple imputation procedure to further assess the influence of missingness and details of the method will be provided in the SAP. Briefly, these methodologies will include multiple imputation under an assumption of missing not at random and tipping-point analyses to explore how extreme the difference between randomized treatments would have to be among subjects with missing data to overwhelm the treatment effect obtained in the primary. In these analyses, the multiple imputation approach will be expanded to allow for varying impact of missing data by incorporating a shift parameter in the imputation model. The range of values for the shift parameter will explore the varying missing data assumptions, including the scenario where the imputation model is completely based on the control group (ie, control-based imputation).
Safety:
All safety analyses will be conducted based on the Safety Analysis Set.
Safety data will include AEs, clinical laboratory tests, vital signs, physical examinations, and ECGs. Observed data will be listed by subject and summarized using descriptive statistics by treatment group.
Pharmacokinetics:
Pharmacokinetic parameters and plasma concentrations will be summarized separately following different PK sampling scheme and population using descriptive statistics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERM

Abbreviation or Specialist Term	Explanation
ACMG	American College of Medical Genetics
AE	Adverse event
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartate Aminotransferase
$AUC_{0\text{-}24h}$	Area under the plasma concentration-time curve from time zero to 24 hours
$AUC_{0\text{-}inf}$	Area under the plasma concentration-time curve from time zero to infinity
BH_4	Tetrahydrobiopterin
BUN	Blood urea nitrogen
CD	Compact Disc
CL/F	Apparent total clearance of the drug from plasma following oral administration
COVID-19	Coronavirus disease 2019
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DBS	Dried blood sample
eCRF	Electronic case report form
EC	Ethics Committee
ECG	Electrocardiogram
EDC	Electronic data capture
EQ-5D	European Quality of Life - 5 Dimensions
EQ-5D-Y	European Quality of Life - 5 Dimensions for Youth
ETV	Early Termination Visit
EU	European Union
FAS	Full Analysis Set
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GGT	Gamma glutamyl transferase
HEENT	Head, eyes, ears, nose, and throat
HPA	Hyperphenylalaninemia
HPLC/MS/MS	High-performance liquid chromatography and mass spectrometry
ICF	Informed Consent Form
IEC	Independent Ethics Committees
IRB	Institutional Review Board
IRT	Interactive Response Technology
LDH	Lactate dehydrogenase
LS	Least squares
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model repeated measures
NDA	New Drug Application
PAH	Phenylalanine hydroxylase
Phe	Phenylalanine
PI	Principal investigator
PK	Pharmacokinetics
PKU	Phenylketonuria
PKU-QOL	Phenylketonuria-quality of life
PP	Per Protocol
QOL	Quality of life
RBC	Red blood cell
RSI	Reference Safety Information
SAE	Serious adverse event

Abbreviation or Specialist Term	Explanation
SAP	Statistical analysis plan
SoC	Standard of care
SOP	Standard Operating Procedure
TEAE	Treatment-emergent adverse event
Tyr	Tyrosine
USB	Universal serial bus
VAMS	Volumetric absorptive microsampling
Vz/F	Apparent volume of distribution during terminal phase after non-intravenous administration
WBC	White blood cell

1. INTRODUCTION

1.1. Phenylketonuria

Phenylketonuria (PKU) is an autosomal-recessive inborn error of metabolism characterized by deficiency of the enzyme phenylalanine hydroxylase (PAH), which metabolizes phenylalanine (Phe) (Scriver 2001). Gene mutations of PAH result in decreased catalytic activity leading to hyperphenylalaninemia (HPA) (Al Hafid 2015). There are many different mutations in the *PAH* gene (>1100), resulting in phenotypic variation in the amount of enzyme produced and/or enzyme activity. Patients with severe forms of PKU, also known as "classical PKU", typically have very high blood phenylalanine (Phe) levels (>1200 µmol/L). A partial deficiency of PAH activity results in a lower degree of blood Phe elevation. High levels of Phe are toxic to the brain and are associated with cognitive dysfunction, memory impairment, and can lead to psychiatric and behavioral problems (Kaufman 1989). If left untreated, severe and irreversible intellectual disability can occur (Scriver 2001, Waisbren 2007). Phenylketonuria is diagnosed at birth with the near universal adoption of newborn screening. Phenylketonuria has been described in all ethnic groups, and its incidence worldwide varies widely, but is estimated to occur in approximately 1 in every 23930 births (Hillert 2020).

1.2. Current Treatment of Phenylketonuria

Currently, there is no cure for PKU. Initial treatment consists of prompt institution of stringent Phe dietary restriction supplemented with specifically designed medical foods. Dietary control is considered the standard of care (SoC). The restriction in protein requires exclusion of natural foods such as meat, fish, milk, cheese, bread, nuts, and many other common food items. Even the intake of vegetables is limited.

The success of dietary control, however, comes at high personal cost to affected individuals and their families. Compliance with a restrictive diet and Phe monitoring can be difficult for older children, adolescents, and adults (Fisch 2000, Walter 2002) and it is accepted that dietary burden does not improve with age (MacDonald 2012). Lifelong management of Phe levels is critical to avoid neurocognitive decline and other comorbidities as recognized by the European Guidance on PKU and the American College of Medical Genetics (ACMG) guidance on PKU (Vockley 2014, van Wegberg 2017). Recently, it has been reported that >60% of adolescents and 70% of adult patients with PKU have uncontrolled blood Phe levels, with concentrations exceeding the upper limit of ACMG target range (360 µmol/L) (Jurecki 2017). The dietary restrictions lead to social difficulties and exclusions, with the potential to make people with PKU feel different and isolated.

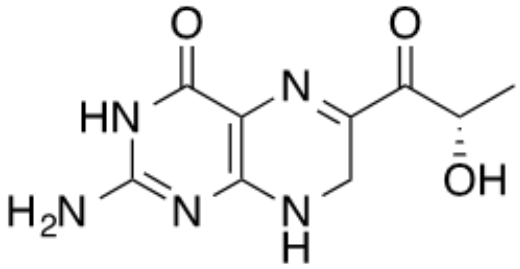
Synthetic tetrahydrobiopterin (BH_4) (eg, sapropterin dihydrochloride), is commercially available as an approved drug for the treatment of HPA in PKU (FDA New Drug Application [NDA] 22181, EMEA/H/C/000943). Sapropterin dihydrochloride is administered orally, once daily at a dose of 5 to 20 mg/kg (KUVAN USPI 2020). During the *Phenylketonuria Scientific Review Conference: State of the Science and Future Research Needs*, international experts concluded that most people with PKU have little or no benefit from sapropterin dihydrochloride, and evidence of long-term clinical improvements was lacking. They further concluded that "*new drugs that are safe, efficacious, and impacting a larger proportion of individuals with PKU are needed*" (Camp 2014).

PALYNZIQ (pegvaliase-pqpz) is a commercially available product that was recently approved for the treatment of adult patients with PKU (aged 16 years and older in the European Union [EU]) who have inadequate blood Phe control (blood Phe levels greater than 600 µmol/L) despite prior management with available treatment options including sapropterin (FDA Biologics License Application 761079, EMEA/H/C/004744). It is a pegylated form of the bacterial derived enzyme, phenylalanine ammonia lyase. While effective at helping lower blood Phe levels to <600 µmol/L, it requires a daily injection and patients in clinical studies experienced significant adverse reactions to PALYNZIQ treatment including anaphylactic reaction, hypersensitivity, anaphylactoid reaction, blood creatine phosphokinase increase, anxiety, arthralgia, serum-sickness, angioedema, hypophenylalaninemia, injection-site reactions, headache, rash, nausea, pruritus, and urticaria, to name a few. PALYNZIQ is not indicated for patients ≤18 years of age in the United States and ≤16 years of age in the EU, and accordingly does not address the unmet need for new medications that are safe and efficacious for children and adolescents (BioMarin 2020).

1.3. PTC923

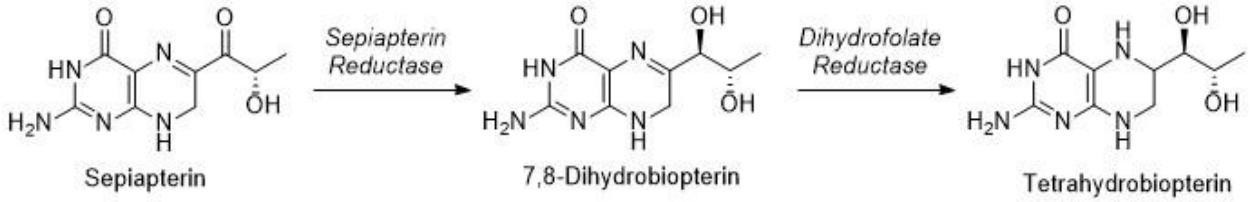
PTC923 (formerly named CNSA-001) is *(S)*-2-amino-6-(2-hydroxypropanoyl)-7,8-dihydropteridin-4*(3H)*-one with a molecular weight of 237.22 g/mol and a molecular formula of $C_9H_{11}N_5O_3$.

The chemical structure of PTC923 is:



PTC923 is a new molecular entity and synthetic form of sepiapterin. Sepiapterin serves as a substrate for de novo synthesis of BH_4 via the pterin salvage pathway (Mayer 1995), making sepiapterin a naturally occurring precursor for BH_4 (Figure 1). Tetrahydrobiopterin is an essential cofactor for enzymes including PAH (Kaufman 1989), tyrosine (Tyr) hydroxylase (Nagatsu 1964), tryptophan hydroxylase (Lovenberg 1967, Ichiyama 1970), fatty acid glycerylether oxygenase (Tietz 1964), and nitric oxide synthase (Kwon 1989, Mayer 1991). Following oral administration, PTC923 is rapidly converted to BH_4 intracellularly (Smith 2019a), the natural cofactor of PAH, and is intended to restore BH_4 to physiological levels in patients who lack endogenous BH_4, increase BH_4 levels in patients who have lower than normal physiological levels of BH_4, or enhance the chaperone effect on PAH in PAH-deficient patients by providing pharmacological levels of BH_4 while also directly enhancing the thermal stability of PAH.

Figure 1: Endogenous Metabolism of BH_4 From Sepiapterin



Abbreviations: BH_4, tetrahydrobiopterin

1.4. Rationale for Study

Currently, there is no cure for PKU. Control with dietary Phe restriction and/or BH_4 supplementation is suboptimal. Tetrahydrobiopterin supplementation with KUVAN has demonstrated clinically meaningful lowering of Phe plasma concentrations in approximately 30% of patients with PKU and was well-tolerated at doses of 5 to 20 mg/kg/day over 22 weeks in BH_4-responsive patients with PKU (Burnett 2007). Further studies showed that the effects on blood Phe are dose-related; however, doses of KUVAN higher than 20 mg/kg/day were not tested (KUVAN NDA 22-181 2007). PTC923 represents the first viable formulation of sepiapterin intended for the treatment of HPA in patients with PKU.

Study PKU-001 was a Phase 1 exploratory study that assessed the safety, tolerability, and pharmacokinetics (PK) of escalating doses of PTC923 administered orally to healthy volunteers. This study showed that PTC923 was safe and well tolerated at single doses of up to 80 mg/kg or following once-daily dosing at doses up to 60 mg/kg for 7 days. Study PKU-001 also provided evidence of increased levels of BH_4 when compared head to head with KUVAN across equivalent doses (Smith 2019b), that would be expected to result in improved efficacy. Following this, Study PKU-002, a Phase 2 study conducted in subjects with PKU, provided a direct head-to-head comparison of PTC923 and KUVAN, with a dose comparison of the effect of 20 mg/kg/day KUVAN versus 20 mg/kg/day PTC923 versus 60 mg/kg/day PTC923. In Study PKU-002, PTC923 60 mg/kg was significantly more effective than KUVAN 20 mg/kg in reducing blood Phe ($p=0.0098$). In addition, comparison of the change in mean blood Phe levels showed that the effect of PTC923 60 mg/kg on blood Phe was approximately 1.4 × larger than that of PTC923 20 mg/kg, driving dose decisions for future studies. There were no notable differences in the safety profile of the PTC923 and KUVAN. Study PKU-002 provided proof of the change in efficacy of PTC923 with statistically superior reductions in blood Phe levels versus KUVAN and helped to establish dose selection for this study.

Study PTC923-MD-003-PKU is a Phase 3 study to assess the efficacy of PTC923 in reducing blood Phe levels in subjects with PKU. It is anticipated that a greater reduction in Phe will be observed in subjects with PKU who receive PTC923 versus those who receive placebo. This Phase 3 study is designed to support registration of PTC923 in subjects with PKU.

1.5. Rationale for Study Design and Control Group

Study PTC923-MD-003-PKU is a double-blind, randomized study that compares PTC923 plus SoC to placebo plus SoC. Standard of care in PKU is a Phe-restricted diet. Aggressive and highly restrictive dietary control is the mainstay of treatment for the majority of patients. Initial and lifelong treatment consisting of prompt institution of Phe dietary restriction supplemented with specifically designed medical foods is the SoC for PKU (van Wegberg 2017). The prestudy Phe-restricted diet for each subject will be specifically determined and monitored in this study to ensure subjects maintain their current Phe-restricted diet throughout the study duration.

The placebo control selected in this study is considered ethically acceptable, practical, and clinically relevant given that the underlining SoC of a Phe-restricted diet will be maintained for all subjects. Given the limited safe and effective pharmacological treatment options available for PKU, placebo control, together with 1:1 randomization, provides an optimal framework to directly assess the efficacy and safety of a new treatment.

During Part 1 (the open-label Responsiveness Test), subjects with PKU will be enrolled and receive PTC923 for 14 days to identify subjects with ≥15% reduction from baseline in blood Phe levels.

Subjects will receive the following doses of PTC923 for 14 days:

- Subjects 0 to <6 months of age - 7.5 mg/kg
- Subjects 6 to <12 months of age - 15 mg/kg
- Subjects 12 months to <2 years of age - 30 mg/kg
- Subjects ≥2 years - 60 mg/kg

Subjects who experience <15% reduction in blood Phe levels will be classified as nonresponsive and will be contacted to schedule an Early Termination Visit (ETV) (on or between Day 28 to Day 35).

Subjects <2 years of age who experience a ≥15% reduction in blood Phe levels will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

PTC923-responsive subjects ≥2 years of age will be entered into Part 2 (the double-blind, randomized Treatment Period). Subjects in the PTC923 treatment arm will receive PTC923 20 mg/kg daily for Weeks 1 and 2, then PTC923 40 mg/kg daily for Weeks 3 and 4, then PTC923 60 mg/kg daily for Weeks 5 and 6. As dosing is weight-based and to maintain the blind, subjects in the placebo arm 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 an open-label extension Study PTC923-MD-004-PKU.

The study inclusion criteria were developed to include the broadest possible criteria to reflect the wider PKU population, while excluding subjects who may be at risk from participation or may confound data interpretation. Subjects must have a clinical diagnosis of PKU with HPA documented by past medical history of at least 2 blood Phe measurements ≥600 µmol/L. Another inclusion criterion specifies subjects must have blood Phe levels ≥360 µmol/L during screening, or when taking the average of the 3 most recent Phe levels from the subject's medical history (inclusive of the Screening value). Setting the values at ≥360 µmol/L allows the inclusion of children, in whom Phe levels above 360 µmol/L would be considered uncontrolled. If the Phe levels were to be set at ≥600 µmol/L, the study would principally enroll adult subjects only. While larger changes in Phe levels may be observed if the starting levels are higher, excluding younger subjects with lower levels of Phe would be short-sighted. Nevertheless, a second stratification will ensure that subjects with baseline blood Phe of <600 µmol/L and subjects with baseline blood Phe ≥600 µmol/L are equally distributed to receive PTC923 or placebo.

1.6. Study Duration

The study duration for each subject will be up to 107 days from Screening to the final study visit. Following a screening period of up to 45 days, all enrolled subjects will receive PTC923 2 weeks followed by a minimum 14-day PTC923 washout period in Part 1. Subjects ≥2 years of age with ≥15% response to PTC923 will continue treatment in Part 2 with either PTC923 or placebo for 6 weeks. Subjects <2 years of age with ≥15% response to PTC923 will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

1.7. Risk/Benefit Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of PTC923 can be found in the current version of the IB and associated reference safety information.

Clinical knowledge exists for PTC923 as it has been evaluated in more than 102 subjects in 4 clinical studies including 24 subjects with PKU. Across all studies, PTC923 has been well tolerated and exhibited a favorable safety profile with manageable side effects. Further, Study PTC923-MD-003-PKU will have a data and safety monitoring board that will closely monitor the safety of subjects.

When developing the inclusion/exclusion criteria and study design for this study, PTC Therapeutics (PTC) identified benefits and risks with mitigation strategies that ensured the safety of subjects and veracity of data collected.

As per inclusion criteria 3, eligible subjects for this study have uncontrolled PKU with blood Phe level ≥360 µmol/L on current therapy anytime during Screening and uncontrolled blood Phe level ≥360 µmol/L on current therapy. PTC923 has been shown to provide statistically significant, dose-related reductions in blood Phe and was faster and more efficacious in reducing blood Phe compared to sapropterin in subjects with uncontrolled PKU (Bratkovic 2022). Further, in subjects with classical PKU, the reduction in mean blood Phe with PTC923 was also larger than that observed with sapropterin.

To minimize exposure to placebo, Part 2 (randomized, placebo-controlled, double-blind treatment period) duration is capped at 6 weeks. This duration permits adequate analysis of efficacy of PTC923 versus placebo but minimizes placebo exposure. During this period, blood Phe levels will be extensively monitored to ensure safety of subjects. No subject aged <2 years will be exposed to placebo.

To reduce exposure of nonresponsive subjects to PTC923, in Part 1, all eligible subjects will be tested for responsiveness to PTC923. Subjects ≥2 years of age who experience <15% reduction in blood Phe levels and 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. To minimize exposure of subjects <2 years of age to placebo, subjects who experience a ≥15% reduction in blood Phe levels will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

During this study, blood Phe will be monitored frequently to assess the responsiveness and efficacy of treatment. PTC has demonstrated that dried blood sample collection using the volumetric absorptive microsampling (VAMS) high-performance liquid chromatography and mass spectrometry (HPLC/MS/MS) method is reliable, repeatable, and the results are comparable to a conventional venous blood sampling human plasma HPLC/MS/MS method. Samples collected in the Phase 2 study (Study PKU-002) demonstrated no bias over the measurable Phe concentration range using VAMS dried blood method. Compared to a standard blood draw (>2 mL) to measure blood Phe, dried blood VAMS uses sample volumes of 10 to 20 µL and produces reliable blood Phe concentration quantification. The VAMS dried blood method allows blood Phe samples to be collected at home in accordance with GCP, a critical advantage given the current coronavirus disease 2019 (COVID-19) pandemic when unpredictable and unplanned closings of clinical sites occur and prevent regular clinical visits or potentially expose patients to unnecessary risks.

Taking into account the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with PTC923 are justified by the anticipated benefits that may be afforded to subjects with PKU.

2. TRIAL OBJECTIVES AND PURPOSE

2.1. Primary Objective

- 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)

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

2.3. Exploratory Objective

- To evaluate changes in blood Tyr overtime, including the Phe:Tyr ratio

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This is a Phase 3, double-blind, randomized, multi-center efficacy study of PTC923 versus placebo in subjects with PKU.

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 Phe levels over the 14-day treatment period for all subjects will be assessed against their pretreatment blood Phe level. 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 non-responsive 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 be 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 participation in the study will be terminated. Following the minimum 14-day PTC923 washout period, all eligible subjects 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 evenly distributed to either PTC923 or placebo treatment arms. 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.

Subjects randomized to PTC923 will receive PTC923 20 mg/kg daily for Weeks 1 and 2, then PTC923 40 mg/kg daily for Weeks 3 and 4, then PTC923 60 mg/kg daily for Weeks 5 and 6. 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 an open-label extension Study PTC923-MD-004-PKU. Approximately 45 centers will participate in this study. The study design is summarized in Figure 2 and the Schedule of Assessments is presented in Table 1 (Screening Period) and Table 2 (Parts 1 and 2).

Throughout the study, subjects will maintain 3-day diet records corresponding to each week of the study. At each in-clinic visit, a dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification (Section 8.1.7).

Virtual visits are acceptable as indicated/or deemed necessary in the Schedule of Assessments (Table 2). However, if study staff feel it necessary and/or appropriate, these visits may be conducted at the study site instead.

3.1.1. Screening Period (Up to 45 Days)

An informed consent and/or assent (as applicable) form must be signed before any study-related procedures are performed. After providing consent and/or assent (as applicable), subjects will undergo in-clinic screening procedures to determine study eligibility. Subject demographics and medical history information will be collected. Additionally, 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). Vital signs, weight, and height measurements will be performed. Phenylalanine hydroxylase gene (*PAH*) genotyping should be performed unless already documented in subject's medical history. Blood Phe/Tyr levels will be measured. Vital signs, weight, and height measurements will be performed. A full physical examination will be performed, and blood samples will be collected for clinical laboratory tests and pregnancy testing. Concomitant medication and AEs will be collected from provision of consent and/or assent (as applicable). Subjects will be instructed to continue their usual diet without modification (ie, no change in daily Phe consumption) throughout the entire study. Subjects will maintain a 3-day diet record during each week of Screening, and a dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, have regular contact with subjects, and reinforce the need for subjects to maintain their usual diet without modification. Subjects who enter the study and are receiving pegvaliase-pqpz supplementation at the Screening Visit must complete a 30-day washout period prior to dosing. Subjects who enter the study and are receiving BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) at the Screening Visit must complete a 7-day washout period prior to dosing.

3.1.1.1. Pegvaliase-pqpz Washout (30 Days)

Following the initial Screening Visit, eligible subjects who are taking pegvaliase-pqpz must discontinue the medication as concomitant treatment/supplementation with pegvaliase-pqpz will not be permitted during the study. Pegvaliase-pqpz will be discontinued 24 hours prior to starting the Washout. Subjects will continue their usual diet without modification (ie, no change in daily Phe consumption) and will maintain a 3-day diet record during the Pegvaliase-pqpz Washout. A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification. Blood Phe levels will be measured at each timepoint for the 30-day washout (Days 1, 20, and 30).

3.1.1.2. BH_4 Supplementation Washout (7 Days)

Following the initial Screening Visit, eligible subjects who are receiving BH_4 supplementation must discontinue the medication as concomitant treatment/supplementation with BH_4 will not be permitted during the study. BH_4 treatment (eg, sapropterin dihydrochloride, KUVAN) will be discontinued 24 hours prior to starting the Supplementation Washout. Subjects will continue their usual diet without modification (ie, no change in daily Phe consumption) and will maintain a 3-day diet record during the BH_4 Supplementation Washout. A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification. Blood will be collected for determination of blood Phe levels at each timepoint for the 1-week washout (Days 1, 4, and 7) using dried blood sampling.

3.1.2. Part 1, Open-Label Responsiveness Test (28 to 35 Days)

3.1.2.1. Part 1, Open-Label Treatment (14 Days)

Following the initial Screening Visit and BH_4 Supplementation/Pegvaliase-pqpz Washout (as necessary), all eligible subjects will be enrolled into Part 1 of the study to test for responsiveness to PTC923. Subjects will receive the following doses of PTC923 for 14 days:

- Subjects 0 to <6 months of age: 7.5 mg/kg
- Subjects 6 to <12 months of age: 15 mg/kg
- Subjects 12 months to <2 years of age: 30 mg/kg
- Subjects ≥2 years: 60 mg/kg

On Part 1 Day 1, subjects will take their dose of PTC923 while in the clinic; for all other dosing days, subjects will take their dose at home and prior to coming to the clinic when visits are scheduled. All visits will be outpatient visits or virtual visits. Study visits will occur on Part 1 Day 1, Part 1 Day 2 (PK substudy only), and Part 1 Day 14 (virtual visit, unless part of the PK substudy).

In Part 1, blood Phe levels will be measured on Days -1, 1 (predose), 2 (PK substudy only), 5, 10, and 14. Baseline blood Phe level will be calculated as the mean of the blood Phe levels from dried blood samples taken on Day -1 and Day 1 (predose). Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.

Subjects will continue their usual diet without modification and will maintain 3-day diet records (ie, one corresponding to each week of the PTC923 open-label treatment period). A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption and to have regular contact with subjects.

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 will 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 the PK su-study to characterize the PK of sepiapterin and BH_4 following an intensive schedule on Part 1 Day 1 and Part 1 Day 2 from subjects ≥2 years. For subjects <2 years of age, 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. Details will be described in the laboratory manual.

3.1.2.2. Part 1, PTC923 Washout (14 Days Minimum)

Following completion of 14 days of PTC923 open-label treatment, subjects will begin a minimum 14-day washout. No in-clinic study visits are required during the PTC923 washout. In Part 1, blood Phe levels will be measured on Days 19, 24, and 28. Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.

Subjects will continue their usual diet without modification and will maintain 3-day diet records (ie, one corresponding to each week of the PTC923 washout). A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification.

During the PTC923 washout, the mean change in blood Phe levels over the 14 days of PTC923 treatment (mean of Part 1 Days 5, 10, and 14) will be measured and compared against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 [predose]). PTC will allow determination of a subject's participation in Part 2 with ≤2 pretreatment blood Phe levels (but requiring a minimum of 1 pretreatment level) and ≤3 PTC923 treatment blood Phe levels (but requiring a minimum of 1 PTC923 treatment level), depending on the available data (on or between Part 1 Study Day 28 to Day 35).

Subjects ≥2 years of age who experience <15% reduction in blood Phe levels will be classified as non-responsive and will be contacted to schedule an ETV (on or between Day 28 to Day 35) (Section 3.1.5). Subjects ≥2 years of age who experience a ≥15% reduction in blood Phe levels will be contacted to confirm their continued eligibility and to schedule the Part 2 Day 1 visit.

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 be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

Part 1 for all subjects ends on Day 35. In no circumstances will a subject be permitted to continue in Part 1 beyond Day 35. If a subject's blood Phe concentrations are not available for determination of responsiveness to PTC923 by Day 35 of Part 1, then that subject will be classified as nonresponsive and will be contacted to schedule an ETV and not be eligible to continue into Part 2 of the study or further participation in an open-label extension Study PTC923-MD-004-PKU.

3.1.3. Part 2, Randomized Double-Blind Treatment Period (42 Days)

All eligible subjects ≥2 years of age will be randomized to receive either PTC923 or placebo for 42 days (ie, 6 weeks) in Part 2, the randomized, placebo-controlled, double-blind treatment period.

Subjects will be stratified based on their mean percent blood Phe reduction in Part 1 (mean % reduction in blood Phe levels of ≥15% to <30% and mean % reduction in blood Phe levels of ≥30%) and evenly distributed across both treatment arms (PTC923 or placebo). 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. This portion of the study is double-blinded. Subjects

will continue their usual diet without modification and will maintain 3-day diet records (one corresponding to each week of Part 2). A dietician will monitor each subject's diet to calculate total protein and corresponding Phe consumption, to have regular contact with subjects, and to reinforce the need for subjects to maintain their usual diet without modification.

Subjects randomized to PTC923 will receive PTC923 20 mg/kg daily for Days 1 to 14 (ie, Weeks 1 and 2), then PTC923 40 mg/kg daily for Days 15 to 28 (ie, Weeks 3 and 4), then PTC923 60 mg/kg daily for Days 29 to 42 (ie, Weeks 5 and 6). 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 used with those randomized to PTC923.

On Part 2 Day 1, subjects will take their dose of study drug (PTC923 20 mg/kg or placebo) in-clinic; for all other dosing days, subjects will take their dose at home and prior to coming to the clinic when visits are scheduled. All visits will be outpatient visits or virtual visits. Study visits will occur on Part 2 Day 1, Part 2 Day 14 (in-clinic visit for PK substudy only), Part 2 Day 28 (in-clinic visit for PK substudy population), and Part 2 Day 42 to conduct study evaluations, collect 3-day diet records, and capture any AEs and concomitant medications.

On Part 2 Day 1 (predose), the Phenylketonuria-quality of life (PKU-QOL) (Parent PKU-QOL [6 to 8 years]; Child PKU-QOL [9 to 11 years]; Adolescent PKU-QOL [12 to 17 years]; Adult PKU-QOL [≥18 years]) will be conducted using the tool in one of the following validated languages: English (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. On Part 2 Day 1 (predose), the European Quality of Life - 5 Dimensions (EQ-5D) (European Quality of Life - 5 Dimensions for Youth [EQ-5D-Y] Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and 5-Level European Quality of Life - 5 Dimensions (EQ-5D-5L) [≥16 years]) will be conducted.

Blood Phe levels will be measured in Part 2 on Days -1, 1 (predose), 5, 10, 14, 19, 24, 28, 33, 38, and 42. Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.

Subjects participating in the PK substudy: 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.

3.1.4. End of Study

For subjects <2 years of age, End of Study is defined as their final visit in Part 1 and should occur on/between Part 1 Day 28 and Part 1 Day 35. All subjects <2 years of age who experience a ≥15% reduction in blood Phe levels during Part 1 will be offered the option to enroll directly into an open-label extension Study PTC923-MD-004-PKU.

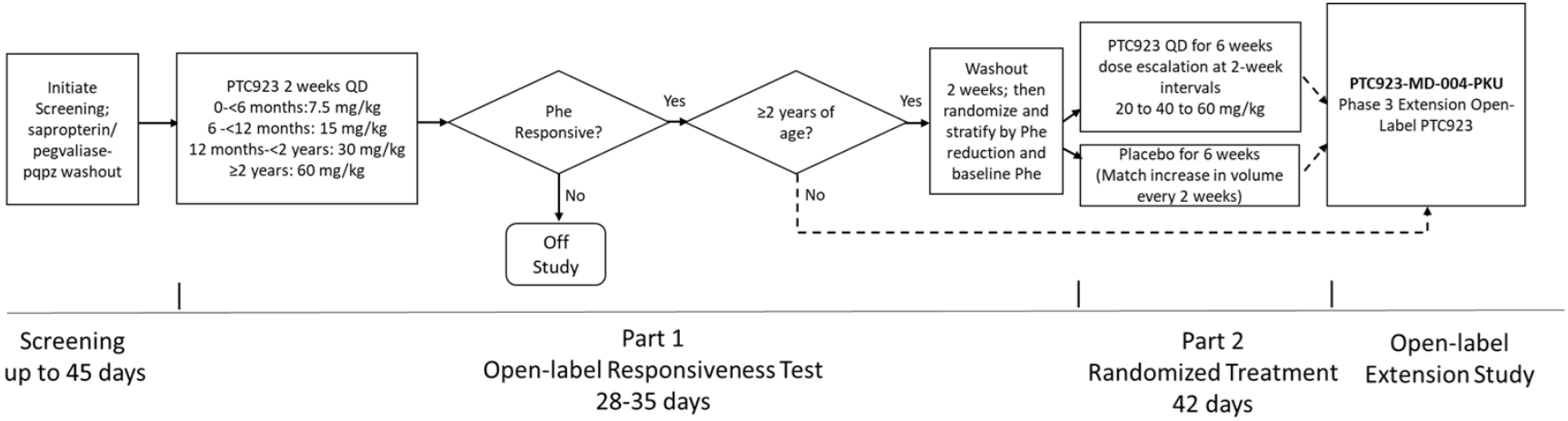
For subjects ≥2 years of age who complete Part 2, End of Study is defined as their final study visit on Part 2 Day 42. All subjects who complete Part 2 will be given the opportunity to continue treatment in the PTC923 Open-Label Extension Study, PTC923-MD-004-PKU.

Enrollment will continue until 80 subjects are randomized into the stratum of ≥30% blood Phe reduction PTC923-responsive subjects. The end of study is defined as the date of the last visit of the last subject in the study globally.

3.1.5. Early Termination Visit

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. During the ETV, 3-day diet records, AEs, concomitant medications, weight, vital signs, ECGs, and blood Phe/Tyr levels will be collected. A physical examination, clinical laboratory tests, and a pregnancy test will be performed. For non-responsive subjects and subjects who discontinue prematurely, following the ETV, subjects may revert to their prestudy treatment for PKU (at the investigators and the subject's treating physician discretion), and a phone follow-up visit will occur 30 (±3) days after the last dose of study drug to assess for serious adverse events (SAEs).

Figure 2: Study Schema for Study PTC923-MD-003-PKU



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

Table 1: 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 (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 performed at the site's local laboratory.

[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 (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 2: 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 [v]
	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[v]	
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 Phe levels (plasma)[q]											X									X	
Blood for PK substudy[r]		X	X			X					X			X			X			X	
Consistent diet/diet monitoring /3-day diet record[s]		X									X										X
QOL Assessments[t]											X										
Dose study drug		Daily for 14 days									Daily for 42 days (6 weeks)										
Dispense study drug		X									X										
Collect study drug, assess compliance						X[u]					X[u]			X[u]			X[u]			X[u]	X[u]

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 cell

[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 Table 17. For all other timepoints, Table 2 should be followed.
[g] To be completed prior to initial dosing and at additional timepoints where indicated (eg, 2-hours postdose 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 Table 18. For all other timepoints, 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 (Section 8.1.5); serum testing to occur at the Screening visit, 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 performed at the site's local laboratory.
[n] Record all treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) starting from 30 days prior to Screening until completion of 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 (predose) 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 (plasma) will be collected for determination of blood Phe levels collected by venipuncture on Part 2 Day 1 predose and Part 2 Day 42 at the time corresponding to the collection of blood Phe/Tyr levels via dried blood sample. Samples will be collected after fasting (eg, Part 2 Day 1 predose) or no earlier than 3 hours postprandial (eg, Part 2 Day 42) and within ±5 minutes of dried blood samples for Phe collection.

[r] 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.

[s] 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.

[t] 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.

[u] 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.

[v] 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.

3.2. Number of Subjects

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.

3.3. Treatment Assignment

3.3.1. Part 1, Open-Label Responsiveness Test

All subjects will receive PTC923 (7.5 mg/kg for subjects 0 to <6 months of age, 15 mg/kg for subjects 6 to <12 months of age, 30 mg/kg for subjects 12 months to <2 years of age, and 60 mg/kg for subjects ≥2 years of age) for 14 days starting on Day 1.

3.3.2. Part 2, Randomized Double-Blind Treatment Period

Eligible subjects will be randomized to receive either PTC923 or placebo for 42 days (6 weeks). Subjects randomized to the PTC923 treatment arm will receive PTC923 20 mg/kg daily for Days 1 to 14 (ie, Weeks 1 and 2), then PTC923 40 mg/kg daily for Days 15 to 28 (ie, Weeks 3 and 4), then PTC923 60 mg/kg daily for Days 29 to 42 (ie, Weeks 5 and 6). 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 those randomized to PTC923.

3.4. Dose Adjustment Criteria

PTC923 is a powder for oral use and dosing is weight based.

Given that PTC923 is packaged in sachets with 2 different dosage strengths, 250 mg and 1000 mg, weight bands will be utilized to determine the sachet size and number required for each subject's dose (Section 6.5).

During Part 1, all subjects will be administered PTC923 orally once a day for 14 days to assess responsiveness; no change of the dose is allowed in this period.

During Part 2, subjects randomized to PTC923 will undergo a forced dose escalation by receiving PTC923 20 mg/kg daily for Weeks 1 and 2, then PTC923 40 mg/kg daily for Weeks 3 and 4, then PTC923 60 mg/kg daily for Weeks 5 and 6. Given the short duration of each dose level, deviation (ie, dose adjustments) from the prescribed dose levels and dose escalation will not be permitted in this study.

3.4.1. Safety Criteria for Adjustment or Stopping Doses

As dose adjustments outside of the prescribed dose escalation scheme are not permitted, any adjustments to or stopping doses of study drug administration would result from concern by the investigator for the safety of the subject. Safety criteria for permanent discontinuation of study drug is discussed in Section 4.4. If a subject temporarily stops treatment at the occurrence of an AE, the subject may return to a previous dose if the investigator and medical monitor feel this is appropriate.

Given the requirement for pregnancy testing in women of childbearing potential at Screening and the requirements for highly effective methods of contraception during the study, it is unlikely that pregnancies will occur during study conduct. However, study drug will be discontinued should suspected or confirmed pregnancy or suspected or confirmed nursing during the study drug administration period occur.

3.5. Criteria for Study Termination

The study may be terminated if significant violations of GCP that compromise the ability to achieve the study objectives or compromise subject safety are observed at any time during the study. With regard to safety, the study may be temporarily suspended or terminated should the investigator, PTC, or Institutional Review Board (IRB)/Independent Ethics Committee (IEC) determine that the safety of study participants is significantly jeopardized. The decision for a temporary or permanent study hold will depend on the nature, frequency, and severity of AEs that were observed in all enrolled subjects to date. In a temporary study hold, no additional subjects will be enrolled into the study or dosed with study drug until the study team members (including the investigator and the medical monitor) decide it is safe to proceed with the study.

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

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

4. SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects with any *PAH* mutation are permitted to screen and enroll into the study. However, subjects with biochemically diagnosed 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 capped at 20% of the total study population. Genotyping will not be required for study eligibility; however, all subjects will undergo genotyping unless documented in their medical history and these data will be collected for analysis.

4.1. Subject Inclusion Criteria

Individuals eligible to participate in this study include subjects who meet all the following criteria:

1. Informed consent and assent (if necessary, at the investigator's discretion [ie, for children and/or subjects that are mentally impaired secondary to disease]) with parental/legal guardian consent
2. Male or female subjects of any age
3. Uncontrolled blood Phe level ≥360 µmol/L on current therapy anytime during Screening and uncontrolled blood Phe level ≥360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the subject's medical history (inclusive of the Screening value)
4. Clinical diagnosis of PKU with HPA documented by past medical history of at least 2 blood Phe measurements ≥600 µmol/L
5. Women of childbearing potential, , as defined in (CTFG 2020), must have a negative pregnancy test at Screening and agree to abstinence or the use of at least one highly effective form of contraception (with a failure rate of <1% per year when used consistently and correctly):
    - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Intravaginal
        - Transdermal
    - Progestogen-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system
    - Bilateral tubal occlusion

- Vasectomized partner with confirmed azoospermia

Highly effective contraception or abstinence must be continued for the duration of the study and for up to 90 days after the last dose of study drug.

All females will be considered of childbearing potential unless they are postmenopausal (at least 12 months consecutive amenorrhea in the appropriate age group without other known or suspected cause) or have been permanently sterilized surgically (eg, hysterectomy, bilateral salpingectomy, bilateral oophorectomy).

6. Males who are sexually active with women of childbearing potential who have not had a vasectomy must agree to use a barrier method of birth control during the study and for up to 90 days after the last dose of study drug. Males must also refrain from sperm donations during this time period.

   Males who are abstinent will not be required to use a contraceptive method unless they become sexually active. Males who have undergone a vasectomy are not required to use a contraceptive method if at least 16 weeks post procedure.

7. Willing and able to comply with the protocol and study procedures
8. Willing to continue current diet unchanged while participating in the study

4.2. Subject Exclusion Criteria

Individuals are not eligible to participate in this study if they have met or meet any of the following exclusion criteria:

1. The individual, in the opinion of the investigator, is unwilling or unable to adhere to the requirements of the study
2. Gastrointestinal disease (such as irritable bowel syndrome, inflammatory bowel disease, chronic gastritis, and peptic ulcer disease, etc.) that could affect the absorption of study drug
3. History of gastric surgery, including Roux-en-Y gastric bypass surgery or an antrectomy with vagotomy, or gastrectomy
4. Inability to tolerate oral medication
5. History of allergies or adverse reactions to synthetic BH_4 or sepiapterin
6. Current participation in any other investigational drug study or use of any investigational agent within 30 days prior to Screening
7. Any clinically significant laboratory abnormality as determined by the investigator. In general, each laboratory value from Screening and baseline chemistry and hematology panels should fall within the limits of the normal laboratory reference range, unless deemed not clinically significant by the investigator
8. A female who is pregnant or breastfeeding, or considering pregnancy
9. Serious neuropsychiatric illness (eg, major depression) not currently under medical control, that in the opinion of the investigator or sponsor, would interfere with the subject's ability to participate in the study or increase the risk of participation for that subject

10. Past medical history and/or evidence of renal impairment and/or condition including moderate/severe renal insufficiency (glomerular filtration rate [GFR] <60 mL/min) and/or under care of a nephrologist
11. Any abnormal physical examination and/or laboratory findings indicative of signs or symptoms of renal disease, including calculated GFR <60 mL/min/1.73m^2.
    In subjects ≥18 years of age, the Modification of Diet in Renal Disease Equation should be used to determine GFR.
    In subjects <18 years, the Bedside Schwartz Equation should be used to determine GFR.
12. Requirement for concomitant treatment with any drug known to inhibit folate synthesis (eg, methotrexate)
13. Confirmed diagnosis of a primary BH_4 deficiency as evidenced by biallelic pathogenic mutations in *6-pyruvoyltetrahydropterin synthase*, recessive *GTP cyclohydrolase I*, *sepiapterin reductase*, *quinoid dihydropteridine reductase*, or *pterin-4-alpha-carbinolamine dehydratase* genes
14. Major surgery within the prior 90 days of screening
15. Concomitant treatment with BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) or pegvaliase-pqpz (PALYNZIQ)
16. Unwillingness to washout from BH_4 supplementation (eg, sapropterin dihydrochloride, KUVAN) or pegvaliase-pqpz (PALYNZIQ)

4.3. Screen Failures

Any subject who does not satisfy inclusion or exclusion criteria within the defined screening window prior to enrollment will be considered a screen failure. Screening and enrollment should occur within a 45-day period. Screen failures will be captured in the electronic data capture (EDC) system. Screen failures can be rescreened after consultation with the medical monitor.

4.4. Subject Withdrawal Criteria

4.4.1. Discontinuation from Study Drug Administration

Premature discontinuation of study drug administration is defined as the discontinuation of study drug for an individual subject before the required full course of study drug is completed. Reasons for premature discontinuation from study drug administration should be recorded on the appropriate page(s) of the electronic case report form (eCRF) and may include, but are not limited to the following:

- Occurrence of an AE, SAE, or clinically significant laboratory abnormality that, in the opinion of the investigator, warrants the subject's permanent discontinuation from study drug administration
- In the judgment of the investigator, the subject experiences a general or specific change(s) that renders the subject unsuitable for continued study drug administration
- There is a need for concomitant medication that makes the subject ineligible for further study drug administration

Adult subjects (ie, ≥18 years) who discontinue from treatment but who do not discontinue from the study will be instructed to maintain adherence to the study schedule of assessments, including all planned safety and efficacy assessments, in the aim of minimizing the risk of missing data (Table 2). After completion of these assessments, the subject should revert to their prestudy SoC to treat PKU (at the discretion of the investigator or the subject's physician). Pediatric subjects (ie, <18 years of age) who discontinue from treatment will not be instructed to maintain adherence to the study schedule of assessments but should revert to their prestudy SoC to treat PKU (at the discretion of the investigator or the subject's physician). In addition, a phone follow-up visit will occur 30 (±3) days after the last dose of study drug to assess for SAEs.

4.4.2. Participant Discontinuation/Withdrawal from the Study

Subjects may withdraw from the study for any reason or be withdrawn at the request of the investigator or sponsor. The reason for a subject's withdrawal must be recorded on the appropriate page(s) of the eCRF. Reasons for withdrawal from the study may include, but are not limited to:

- Withdrawal of consent and/or assent (as applicable)
- AEs or SAEs
- Significant subject noncompliance, defined as refusal or inability to adhere to the protocol requirements
- The investigator determines that it is in the best interest of the subject to withdraw from study participation

Each subject who withdraws from the study after receipt of any amount of study drug will be asked to undergo the ETV assessments. However, subjects may withdraw consent to participate in this study at any time without penalty. Withdrawn subjects will not be replaced.

4.5. Lost to Follow-Up

A subject is considered lost to follow-up if he or she does not complete the study and attempts to contact the subject are unsuccessful. Efforts must be made on the part of the site to avoid any subject being lost to follow-up during the study. Before any subject is considered lost to follow-up, a minimum of 2 documented telephone contact attempts and 1 certified letter within a week of the most recent planned study assessment must be sent in efforts to contact the subject. After being considered lost to follow-up, a subject's status may be changed if the subject makes contact at a later time, provided that the study is ongoing.

5. TREATMENT OF SUBJECTS

5.1. Description of Study Drug

PTC923 is a new chemical entity and synthetic form of sepiapterin. Sepiapterin is *(S)*-2-amino-6-(2-hydroxypropanoyl)-7,8-dihydropteridin-4*(3H)*-one with a molecular weight of 237.22 g/mol and a molecular formula of $C_9H_{11}N_5O_3$.

PTC923 Powder for Oral Use is a powder for oral use with a yellow to orange colour. PTC923 Powder for Oral Use will be suspended in water or apple juice prior to administration. Additional details on the study drug are included in Section 6.

5.2. Selection and Timing of Dose for Each Subject

PTC923 will be dispensed at Part 1 Day 1 and Part 2 Day 1 in-clinic visits. The first dose in each treatment period will be taken in the clinic with a meal; for all other dosing days, subjects will take their morning dose as an outpatient with a meal.

For subjects participating in the PK substudy, if an in-clinic visit is scheduled for PK blood sampling, doses will be administered in-clinic with a meal.

5.3. Concomitant Medications

Concomitant use of any drugs known to inhibit folate synthesis (eg, methotrexate, pemetrexed, and trimetrexate), pegvaliase-pqpz, BH_4 (eg, sapropterin dihydrochloride, KUVAN), or any investigational therapy will not be permitted.

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 on the eCRF. Additionally, 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). During Screening, each subject will be instructed to report the use of any medication to the investigator. Subjects will also be instructed about the importance of not taking any medication throughout the study (including over-the-counter medications) without consulting the investigator.

COVID-19 vaccination is permitted during the study and subjects are encouraged to be vaccinated according to local guidelines. Given the mode of action of PTC923, it is considered unlikely there would be an interaction/immune response between PTC923 and the COVID-19 vaccination. Vaccination should be reported to the investigator in-line with standard concomitant medication reporting.

5.4. Total Blood Volume

The total volume of blood that will be collected from each subject for Screening, Part 1, and Part 2 of the study is presented in Table 3, Table 4, and Table 5, respectively. Blood volumes taken are in accordance with current guidance that recommends a blood draw limit of 1% of total blood volume corresponding to a single time point and 3% of total blood volume during a period of four weeks (European Parliament 2008). Total blood volume is estimated to be 80 to 90 mL/kg body weight (100 mL/kg body weight for neonates). Subjects of all ages are permitted for inclusion into this study. Based on growth charts for newborns, 5% to 95% of boys weigh 2.6

to 4.2 kg and girls weigh 2.5 to 4.0 kg (CDC 2010a, CDC 2010b). Accordingly, the maximum total blood volume permitted for newborns would be 2.5 mL at a single time and 7.5 mL over 4 weeks. Based on growth charts for subjects 2 years old, 5% to 95% of boys weigh 10.6 to 15.2 kg and girls weigh 10.2 to 14.6 kg (CDC 2010a, CDC 2010b). Accordingly, the maximum total blood volume permitted for subjects 2 years old would be 8.2 mL at a single time and 24.5 mL over 4 weeks. To adhere to the guidance, three groups are represented with corresponding blood volumes in Table 3 and Table 4. Table 5 only includes 2 groups as only subjects ≥2 years of age will participate in Part 2 of the study.

Screening, consisting of the Screening visit and Supplementation Washout Period, may last up to 45 days (6 weeks). If a subject weighs less than 4.5kg and *PAH* genotyping is required, then *PAH* genotyping should be performed on a separate day from the collection of blood chemistry, hematology, vitamin B12/iron, and blood Phe/Tyr levels.

Table 3: Blood Volume – Screening (Screening Visit and Supplementation Washout Period)

	Subjects ≥12 Years		**Subjects ≥2 years <12 Years**		**Subjects <2 Years**	
	Vol × Frequency	**Total Vol**	**Vol × Frequency**	**Total Vol**	**Vol × Frequency**	**Total Vol**
Blood chemistry	5.0 mL×1	5.0 mL	1.2 mL×1	1.2 mL	1.2 mL×1	1.2 mL
Hematology	3.0 mL×1	3.0 mL	1.2 mL×1	1.2 mL	1.2 mL×1	1.2 mL
Vitamin B12/iron (incl. serum pregnancy test in female subjects [if applicable])	3.5 mL×1	3.5 mL				
Blood Phe and Tyr levels	0.12 mL×1	0.12 mL	0.12 mL×1	0.12 mL	0.12 mL×1	0.12 mL
PAH genotyping [a]	2.0 mL×1	2.0 mL	2.0 mL×1	2.0 mL	2.0 mL×1	2.0 mL
Blood Phe and Tyr levels during supplementation washout	0.12 mL×3	0.36 mL	0.12 mL×3	0.36 mL	0.12 mL×3	0.36 mL
Total Blood Volume		**13.98 mL**		**4.88 mL**		**4.88 mL**

Abbreviations: Phe, phenylalanine; Tyr, tyrosine
[a] Only required if genotyping is not part of PKU medical history (Section 8.1.9)

Part 1, consisting of the open-label PTC923-responsiveness test and PTC923 washout period, may last up to 35 days (5 weeks).

Table 4: Blood Volume – Part 1 (Open-Label Responsiveness Test Period)

	Subjects ≥12 Years		**Subjects ≥2 years <12 Years**		**Subjects <2 Years**	
	Vol × Frequency	**Total Vol**	**Vol × Frequency**	**Total Vol**	**Vol × Frequency**	**Total Vol**
Blood chemistry	5.0 mL×1	5.0 mL	1.2 mL×1	1.2 mL	1.2 mL×1	1.2 mL
Hematology	3.0 mL×1	3.0 mL	1.2 mL×1	1.2 mL		
Vitamin B12/iron (incl. serum pregnancy test in female subjects [if applicable])	3.5 mL×1	3.5 mL			1.2 mL×1	1.2 mL
Blood Phe and Tyr levels	0.12 mL×8	0.96 mL	0.12 mL×8	0.96 mL	0.12 mL×8	0.96 mL
Total Blood Volume		**12.46 mL**		**3.36 mL**		**3.36 mL**
Additional blood volume for subjects participating in PK substudy						
PTC923 PK samples (PK subpopulation)	2.0 mL×8	16.0 mL	0.5 ml ×8	4.0 mL	0.5 mL×4	2.0 mL
Phe/Tyr from PK samples (PK subpopulation)	0.25 mL×8	2.0 mL	0.25 mL×8	2.0 mL	0.25 mL×4	1 mL
Total Blood Volume		**30.46 mL**		**9.36 mL**		**6.36 mL**

Abbreviations: Phe, phenylalanine; PK, pharmacokinetics; Tyr, tyrosine

Part 2, consisting of the randomized double-blind treatment period may last up to 42 days (6 weeks).

Table 5: Blood Volume – Part 2 (Randomized Double-Blinded Treatment Period)

	Subjects ≥12 Years		Subjects ≥2 years <12 Years	
	Vol × Frequency	**Total Vol**	**Vol × Frequency**	**Total Vol**
Blood chemistry	5.0 mL×2	10.0 mL	1.2 mL×2	1.2 mL
Hematology	3.0 mL×2	6.0 mL	1.2 mL×2	1.2 mL
Vitamin B12/iron (incl. serum pregnancy test in female subjects [if applicable])	3.5 mL×2	7.0 mL		
Blood Phe and Tyr levels	0.12 mL×11	1.32 mL	0.12 mL×11	1.32 mL
Blood plasma Phe levels	2.0 mLx2	4.0 mL	2.0 mLx2	4.0 mL
Total Blood Volume		**28.32 mL**		**10.12 mL**
Additional blood volume for subjects participating in PK substudy				
PTC923 PK levels (PK subpopulation)	2.0 mL×8	16.0 mL	0.5 mL×8	4.0 mL
Total Blood Volume		**44.32 mL**		**14.12 mL**

Abbreviations: ETV, Early Termination Visit; Phe, phenylalanine; PK, pharmacokinetics; Tyr, tyrosine
Note: If an ETV is conducted, depending on timing, an additional blood chemistry sample will be taken.

5.5. Treatment Compliance

Study drug will be dispensed on Day 1 of each treatment period. Subjects will be instructed to return all used (empty) and unused study drug sachets at each scheduled study visit after Part 1 Day 1. Compliance with the dosing regimen will be assessed by reconciliation of used and unused study drug. The quantities dispensed, returned, used, and lost will be recorded on the dispensing log provided for the study.

5.6. Randomization and Blinding

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 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 evenly distributed to either PTC923 or placebo treatment arms. 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.

Subjects who are unblinded could potentially remain in the study, per principal investigator (PI) discretion and based on discussions with medical monitor as needed.

5.6.1. Measures to Minimize Bias: Randomization and Blinding

Randomization is an accepted means to reduce bias and allows for the highest standard of evidence in documenting a treatment effect. The block randomization technique will be used. The process will be established and performed centrally by an experienced Contract Research Organization (CRO) through an Interactive Response Technology (IRT) 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: however, the blind may be broken in the event of a medical emergency, in which knowledge of the study drug identity is critical to the management of the subject's course of treatment. Before breaking the blind, the investigator should determine that the information is necessary (ie, that it will alter the subject's immediate course of treatment). In many cases, particularly when the emergency is clearly not study -drug related, the problem may be effectively managed by assuming that the subject is receiving an active study drug without the need for unblinding. If deemed necessary to break the blind for a study subject, the investigator has the final decision and unilateral right to break the blind and should notify the PTC medical monitor as soon as possible after breaking the blind for a subject.

To identify a subject's study drug, the investigator or designee must electronically contact the IRT system to identify a subject's study drug assignment. Record the date of unblinding, the reason for unblinding, and the initials of the investigational center staff member who performed the unblinding on the subject's study file.

Subjects who are unblinded could potentially remain in the study, per PI discretion and based on discussions with medical monitor as needed.

Instructions for use of the IRT system will be provided to each investigator prior to the initiation of subject enrollment at her/his center.

6. STUDY DRUG MATERIALS AND MANAGEMENT

6.1. Study Drug

The test product is PTC923 Powder for Oral Use. The excipients include microcrystalline cellulose, isomalt, mannitol, croscarmellose sodium, xantham gum, colloidal silicon dioxide, sucralose, and magnesium stearate. PTC923 will be suspended in water or apple juice prior to administration. PTC923 is packaged in aluminum foil sachets. Each sachet contains either 250 mg or 1000 mg of PTC923.

Placebo for PTC923 Powder for Oral Use is also provided as a powder for oral use and is packaged in aluminum foil sachets to match PTC923 in appearance. The excipients include microcrystalline cellulose, isomalt, mannitol, croscarmellose sodium, xantham gum, colloidal silicon dioxide, sucralose, and colorants, tartrazine (soluble) and tartrazine (insoluble). Placebo for PTC923 Powder for Oral Use will be suspended in water or apple juice prior to administration. As Placebo for PTC923 Powder for Oral Use is to be used only in Part 2, subjects assigned to the placebo treatment arm during Part 2 will be given equivalent quantities of placebo to maintain the blind.

6.2. Study Drug Dispensing

Study drug will be dispensed as shown in Table 2. A sufficient supply of study drug will be dispensed for dosing each time (with additional days provided to account for lost or damaged product). Details on the preparation of study drug, dosing guidelines, and storage will be provided in a pharmacy manual for the site and an instruction guide for subjects.

6.3. Study Drug Packaging and Labeling

PTC923 Powder for Oral Use is packaged in heat-sealed aluminum foil sachets that are available in 2 strengths. Each sachet contains either 250 mg or 1000 mg of PTC923.

PTC923 Powder for Oral use and Placebo for PTC923 for Oral Use are packaged in heat-sealed aluminum foil sachets. A kit will contain 30 sachets.

All kits and sachets will be labelled in accordance with local regulatory requirements.

6.4. Study Drug Storage

PTC923 Powder for Oral Use should be stored at refrigerated conditions (2 to 8^{o}C). Constituted PTC923 suspension should be administered immediately. During the time from constitution to administration, the resulting suspension may be stored at room temperature.

Placebo for PTC923 Powder for Oral Use should be stored in refrigerated conditions (2 to 8^{o}C). Constituted Placebo for PTC923 Powder for Oral Use should be administered immediately. During the time from constitution to administration, the resulting suspension may be stored at room temperature.

6.5. Study Drug Preparation

As dosing is weight-based, subjects will receive kits containing the required number of sachets for each 2-week period. Study drug will be provided to each subject based on their current/most recent weight, age, and randomization sequence (as applicable) such that subjects will be informed about how many sachets to use for each dose (eg, 3 large sachets and 1 small sachet, or 2 large sachets and 3 small sachets).

PTC923 Powder for Oral Use is provided as 250 mg and 1000 mg sachets. The calculated daily dose on body weight should be rounded to the nearest multiple of 250 mg or 1000 mg, as appropriate. For instance, a calculated dose of 1251 to 1374 mg should be rounded down to 1250 mg corresponding to 1×250 mg sachet and 1×1000 mg sachet. A calculated dose of 1375 to 1499 mg should be rounded up to 1500 mg corresponding to 2×250 mg sachets and 1×1000 mg sachet.

The full contents of a single 250 mg or 1000 mg sachet may be suspended in 10 mL or 20 mL of water or apple juice, respectively. Once added, the combination should be shaken or stirred for at least 30 seconds to form the suspension. After mixture, all of the resulting suspension or an appropriate quantity of the resulting suspension depending on the weight of the individual should be measured and administered PO to the subject immediately. Dosing is weight based on 1 mg of PTC923 per kg of subject basis.

Subjects will be instructed to open their study drug kit and remove the required number of sachets when preparing their daily dose.

For subjects ≥2 years of age weighing ≤18 kg administering 20 mg/kg dose

For subjects ≥2 years of age weighing ≤18 kg, PTC923 Powder for Oral Use should be suspended in 9 mL of water or apple juice per 250 mg sachet and an aliquot of this suspension corresponding to a 20 mg/kg dose may be administered orally via an oral dosing syringe. Table 6 provides dosing information for subjects at 20 mg/kg doses. A graduated dosing syringe will be provided for subjects weighing ≤18 kg for this dose.

Table 6: 20 mg/kg/day Dosing Table for Subjects ≥2 Years of Age Weighing ≤18 kg

Subject Weight (kg)[a]	Target Dose: 20 mg/kg/day[b]			
	Dose (mg)	PTC923 or Placebo for PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
8	160	1	9	6.4
9	180	1	9	7.2
10	200	1	9	8
12	240	1	9	9.6
12.5	250	1	9	10
15	300	2	18	12
18	360	2	18	14.4

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 20 mg/kg/day for subjects.
[c] PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.

For subjects ≥2 years of age weighing ≤15 kg administering 40 and 60 mg/kg doses

For subjects ≥2 years of age weighing ≤15 kg, PTC923 Powder for Oral Use should be suspended in 9 mL of water or apple juice per 250 mg sachet and an aliquot of this suspension corresponding to a 40 and 60 mg/kg dose may be administered orally via an oral dosing syringe. Table 7 and Table 8 provide dosing information for subjects at 40 and 60 mg/kg doses, respectively. A graduated dosing syringe will be provided for subjects weighing ≤15 kg for these 2 doses.

Table 7: 40 mg/kg/day Dosing Table for Subjects ≥2 Years of Age Weighing ≤15 kg

Subject Weight (kg)[a]	Target Dose: 40 mg/kg/day[b]			
	Dose (mg)	PTC923 or Placebo for PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
8	320	2	18	12.8
9	360	2	18	14.4
10	400	2	18	16
12	480	2	18	19.2
12.5	500	2	18	20
15	600	3	27	24

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 40 mg/kg/day for subjects.
[c] PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.

Table 8: 60 mg/kg/day Dosing Table for Subjects ≥2 Years of Age Weighing ≤15 kg

Subject Weight (kg)[a]	Target Dose: 60 mg/kg/day[b]			
	Dose (mg)	PTC923 or Placebo for PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c,f]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
8	480	2	18	19.2
9	540	3	27	21.6
10	600	3	27	24
12	720	3	27	28.8
12.5	750	3	27	30
15	900	1 × 1000 mg sachet	36	36

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 60 mg/kg/day for subjects.
[c] PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg, or Placebo for PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.
[f] Use 1000 mg sachets when indicated.

For subjects <6 months of age weighing ≤12 kg administering 7.5 mg/kg dose

For subjects <6 months of age weighing ≤12 kg, PTC923 Powder for Oral Use should be suspended in 9 mL of water or apple juice per 250 mg sachet and an aliquot of this suspension corresponding to a 7.5 mg/kg dose may be administered orally via an oral dosing syringe. Table 9 provides dosing information for subjects <6 months at 7.5 mg/kg dose. A graduated dosing syringe will be provided for subjects weighing ≤12 kg for this dose.

Table 9: 7.5 mg/kg/day Per Day Dosing Table for Subjects <6 Months of Age Weighing ≤12 kg

Subject Weight (kg)[a]	Target Dose: 7.5 mg/kg/day[b]			
	Dose (mg)	PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
1	7.5	1	9	0.3
1.2	9	1	9	0.36
1.4	10.5	1	9	0.42
1.7	12.8	1	9	0.51
2	15	1	9	0.6
2.5	18.8	1	9	0.75
3	22.5	1	9	0.9
3.5	26.3	1	9	1.05
4	30	1	9	1.2
4.5	33.8	1	9	1.35
5	37.5	1	9	1.5
6	45	1	9	1.8
7	52.5	1	9	2.1
8	60	1	9	2.4
9	67.5	1	9	2.7
10	75	1	9	3
12	90	1	9	3.6

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 7.5 mg/kg/day for subjects
[c] PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.

For subjects ≥6 to <12 months of age weighing ≤15 kg administering 15 mg/kg dose

For subjects ≥6 to <12 months of age weighing ≤15 kg, PTC923 Powder for Oral Use should be suspended in 9 mL of water or apple juice per 250 mg sachet and an aliquot of this suspension corresponding to a 15 mg/kg dose may be administered orally via an oral dosing syringe. Table 10 provides dosing information for subjects ≥6 to <12 months of age at 15 mg/kg dose. A graduated dosing syringe will be provided for subjects weighing ≤15 kg for this dose.

Table 10: 15 mg/kg/day Dosing Table for Subjects ≥6 to <12 Months of Age Weighing ≤15 kg

Subject Weight (kg) [a]	Target Dose: 15 mg/kg/day[b]			
	Dose (mg)	PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
4	60	1	9	2.4
4.5	67.5	1	9	2.7
5	75	1	9	3
6	90	1	9	3.6
7	105	1	9	4.2
8	120	1	9	4.8
9	135	1	9	5.4
10	150	1	9	6
12	180	1	9	7.2
15	225	1	9	9

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 15 mg/kg/day for subjects ≥6 months but <12 months.
[c] PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.

For subjects ≥12 months to <2 years of age weighing ≤18 kg administering 30 mg/kg dose

For subjects ≥12 months to <2 years of age weighing ≤18 kg, PTC923 Powder for Oral Use should be suspended in 9 mL of water or apple juice per 250 mg sachet and an aliquot of this suspension corresponding to a 30 mg/kg dose may be administered orally via an oral dosing syringe. Table 11 provides dosing information for subjects ≥12 months to <2 years of age at 30 mg/kg dose. A graduated dosing syringe will be provided for subjects weighing ≤18 kg for this dose.

Table 11: 30 mg/kg/day Dosing Table for Subjects ≥12 Months to <2 Years of Age Weighing ≤18 kg

Subject Weight (kg)[a]	Target Dose: 30 mg/kg/day[b]			
	Dose (mg)	PTC923 or Placebo for PTC923 Powder for Oral Use, 250 mg Sachets Suspended[c]	Dilution Volume (mL)[d]	Administered Dose Volume (mL)[e]
5	150	1	9	6
6	180	1	9	7.2
7	210	1	9	8.4
8	240	1	9	9.6
9	270	2	18	10.8
10	300	2	18	12
12	360	2	18	14.4
15	450	2	18	18
18	540	3	27	21.6

[a] Round the subject's weight to the nearest listed weight in the table in kg. For instance, a subject who weighs between 2.01 and 2.24 kg should be rounded down to 2 kg and a subject who weighs between 4.75 and 4.99 kg should be rounded up to 5 kg.
[b] Target dose of 30 mg/kg/day for subjects.
[c] PTC923 Powder for Oral Use, 250 mg provided in single use sachets.
[d] Volume of liquid (water or apple juice) to suspend PTC923 Powder for Oral Use, 250 mg.
[e] Discard remainder of mixture after volume to be administered is drawn.

6.6. Administration

During Part 1, all subjects will receive PTC923 administered orally once a day for 14 days.

During Part 2, subjects in the PTC923 treatment arm will receive PTC923 20 mg/kg for Weeks 1 and 2, then PTC923 40 mg/kg for Weeks 3 and 4, then PTC923 60 mg/kg for Weeks 5 and 6. As dosing is weight-based and to maintain the blind, subjects in the placebo arm will receive equivalent quantities of placebo to match the 20 to 40 to 60 mg/kg dose escalation of the PTC923 treatment arm.

All doses will be administered orally, once a day with food and are weight based. Subjects will be instructed to administer each dose immediately upon reconstitution of the powder into a suspension by drinking the entire contents of the suspension. Subjects will be instructed to rinse the glass with additional water or apple juice and drink the rinse to ensure that the full dose has been administered. In the case of subjects who weigh ≤18 kg or 15 kg (dose level-dependent), the volume administered will be prescribed and administration will be completed via a dosing syringe (Section 6.5).

If a subject vomits after taking a dose of study drug, this should be recorded as an AE and the subject should wait until the next scheduled timepoint to administer a dose. A missed dose should be taken as soon as possible, but 2 doses should not be taken on the same day.

6.7. Study Drug Accountability

All drug product required for completion of this study will be provided by PTC. It is the responsibility of the pharmacy staff or study staff to ensure that a current record of drug inventory and drug accountability is maintained. Inventory and PTC923 accountability records must be readily available for inspection by the study monitor and are open to inspection at any time by applicable regulatory authorities.

6.8. Study Drug Handling and Disposal

Used and unused clinical supplies must be returned to PTC or its designee after the study is completed. If the Standard Operating Procedure (SOP) at any site states that the drug cannot be returned and must be disposed of onsite, PTC must review the SOP of that site prior to any final disposition done by site. Records documenting the date of study drug destruction or shipping, and amount destroyed or shipped should be kept.

7. ASSESSMENT OF EFFICACY

7.1. Primary Efficacy: Phenylalanine Concentrations

The ACMG guidelines recommend lifelong treatment of PKU, with the primary goal of therapy to lower blood Phe to 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 HPA due to PKU. The reliability of blood Phe levels as a predictive biomarker of clinical outcomes in the development of treatments for PKU was assessed in a systematic literature review and meta-analysis of published trials of PKU, which included Phe level and neurological and dietary compliance outcome measures. Within-study correlations between Phe level and IQ were extracted from 40 studies. Significant, proportional correlations were found during critical periods (from 0 to 12 years of age) for early-treated patients with PKU (r=-0.35; 95% CI: -0.44 to -0.27), where each 100 µmol/L increase in Phe predicted a 1.3- to 3.1-point reduction in IQ. Similar significant correlations were observed between IQ and mean lifetime Phe level for early-treated patients (r=0.34; 95% CI: -0.42 to -0.25), where each 100 µmol/L increase in Phe predicted a 1.9- to 4.1-point reduction in IQ. Moderate correlations were found between concurrent Phe level and IQ for early-treated patients. In conclusion, these results confirm a significant correlation between blood Phe level and IQ in patients with PKU and support the use of Phe as a predictive biomarker for IQ in clinical trials (Waisbren 2007).

Furthermore, there is clinical and regulatory precedent for the use of this surrogate clinical efficacy endpoint, which was accepted for both KUVAN and PALYNZIQ in global marketing authorizations (Levy 2007, Harding 2018, Thomas 2018).

The primary efficacy endpoint evaluates the absolute reduction in Phe levels at the optimal dose of PTC923. Using the average over a 2-week period reduces the impact of variations between weeks. It is well established that blood Phe decreases during the day, with the highest blood Phe levels presenting early in the morning following an overnight fast (van Wegberg 2017).

Blood collection, processing, and shipping information will be provided in a laboratory manual.

Blood Phe levels will be measured at the timepoints indicated in Table 12 and Table 13. 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. Samples collected on Day -1 and Day 1 (predose) for Part 1 and Part 2 of each treatment period should be collected predose. At other timepoints, samples can be collected pre-or post-dose.

Samples for blood Phe levels measurement will be collected while the subject is either in the clinic or at home, depending on whether the subject has a scheduled clinic visit. Dried blood samples obtained at home will be shipped to the study site. Samples will be collected using the VAMS technology by utilizing the Mitra microsampling devices provided by Neoteryx (CA, USA) and blood Phe level will be measured using an HPLC/MS/MS method. The method has been validated. Dried blood VAMS provides convenience to the subjects, allowing blood samples (20 µL per sample) to be collected at home, without clinical visits, and samples are shipped at room temperature. When subjects provide samples in clinic, site staff can provide additional training on the sampling technique if considered necessary or requested. Analysis of the blood Phe value from Screening can be performed at the site's local laboratory.

Table 12: 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 13: 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
Plasma[c]											X									X

Abbreviations: DBS, dried blood sample; ETV, Early Termination Visit; Phe, phenylalanine; 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 predose. All other samples can be collected predose or postdose.

[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.

[c] Phe only. Samples will be collected after fasting (eg, Part 2 Day 1 predose) or no earlier than 3 hours postprandial (eg, Part 2 Day 42) and within ±5 minutes of dried blood samples for Phe collection.

7.2. Exploratory Efficacy: Tyrosine Concentrations

Blood collection, processing, and shipping information will be provided in a laboratory manual.

Blood Tyr levels via dried blood samples will be measured at the timepoints indicated in Table 12 (Part 1) and Table 13 (Part 2). 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.

Samples for blood Tyr levels measurements will be collected while the subject is either in the clinic or at home, dependent on whether the subject has a scheduled clinic visit. Dried blood samples obtained at home will be shipped to the study site. Samples will be collected using the VAMS technology by utilizing the Mitra microsampling devices provided by Neoteryx (CA, USA) and blood Phe levels will be measured using an HPLC/MS/MS method. The method has been validated. Dried blood VAMS provides convenience to the subjects, allowing blood samples (20 µL per sample) to be collected at home, without clinic visits, and samples are shipped at room temperature. When subjects provide samples in clinic, site staff can provide additional training on the sampling technique if considered necessary or requested.

8. ASSESSMENT OF SAFETY

8.1. Safety Parameters

8.1.1. Demographic/Medical History

A detailed medical/surgical history will be obtained at Screening. The history will include specific information related to any prior or existing medical conditions or surgical procedures involving the following systems: dermatologic; head, eyes, ears, nose, and throat (HEENT); lymphatic; cardiovascular; respiratory; gastrointestinal; musculoskeletal; neurological; and skin. Additionally, 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).

Newborn Phe concentrations and the 3 most recent Phe concentrations will be collected.

Demographic data will include age, gender, and self-reported race/ethnicity.

8.1.2. Vital Signs, Weight, and Height

Vital signs assessments will include blood pressure, pulse, respiratory rate, and temperature. Vital signs should be obtained 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 at the Screening Visit, Part 1 Day 1, Part 2 Days 1 and 42, and 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 prior to laboratory sampling.

Weight will be collected at the Screening Visit, Part 1 Day 1, Part 2 Days 1 and 42, and ETV (if applicable). Measurements to be completed prior to initial dosing for Part 1 Day 1 and Part 2 Day 1. Weight can be measured at any time during the other visits.

Height will be collected at the Screening Visit. Additionally, for subjects <18 years of age, height should be measured at Part 2 Day 42 (EOS) Visit and at any Unscheduled Visits when eGFR calculation is desired.

Permitted time windows are presented in Table 2 and Table 17 (PK sampling days).

8.1.3. 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, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, neurological, and skin parameters will be assessed.

8.1.4. Electrocardiogram

Twelve-lead electrocardiograms (ECGs) will be obtained before any other study-related procedures are performed. If feasible, ECGs will be performed after a 5-minute rest in the supine position. Twelve-lead ECGs are to be performed at Part 1 Day 1 (predose) for all subjects and at the ETV (if applicable). Additional 12-led ECGs will be performed for all subjects participating in the 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.

Permitted time windows are presented in Table 2 and Table 18 (PK sampling days).

The following parameters will be collected and recorded in the eCRF: RR interval, PR interval, QRS interval, QT interval, and QTc interval. In addition, the tracing should be reported as normal, abnormal clinically significant, or abnormal not clinically significant. If abnormalities are noted on the ECG, these should be recorded in the eCRF.

8.1.5. Laboratory Assessments

Blood and urine samples for clinical chemistry, hematology, and urinalysis will be collected at the timepoints in Table 1 and Table 2. 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 prior to sample collection). Serum and urine pregnancy tests are required for all women of childbearing potential; serum testing to occur during the initial 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 performed at the site's local laboratory.

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

Additionally:

- at the EOS Visit, eGFR should be calculated (all subjects).
- at any Unscheduled Visits in which eGFR calculation is desired serum creatinine should be measured.

8.1.6. Laboratory Abnormalities

Laboratory values will be collected throughout the study to assess for safety. The investigator must review and assess all laboratory results in a timely manner and determine whether the abnormal laboratory values, if any, are clinically significant or not clinically significant and whether there are associated signs and symptoms. The investigator must make the determination whether the clinically significant abnormal laboratory values are AEs. An abnormal laboratory finding in absence of any other signs or symptoms is not necessarily an AE. If the abnormal laboratory finding is accompanied by signs or symptoms, report the signs and symptoms as the AE in lieu of the abnormal laboratory value. If a diagnosis is available, report the diagnosis. Clinically significant laboratory abnormalities after taking study medication that reflect a meaningful change from the screening value(s) and that require active management are to be considered by the investigator as AEs (eg, abnormalities that require study treatment dose modification, discontinuation, more frequent follow-up assessments, etc).

8.1.7. Diet Monitoring: 3-Day Diet Records

Subjects will be instructed to continue their usual diet without modification (ie, no change in daily Phe consumption). Subjects will maintain 3-day diet records during the study as indicated in Table 1 and Table 2. The 3-day diet records will be completed over 3 consecutive days within each period and collected weekly. At each in-clinic visit, a dietician will monitor each subject's diet to calculate Phe consumption and to have regular contact with the subjects. The total Phe consumption during the 3-day period will be calculated by the dietician and entered in the eCRF.

8.1.8. Pharmacokinetic Assessments

Pharmacokinetic sample collection, processing, storage, and shipment will be performed according to instructions outlined in the PK laboratory manual separate from this protocol. Additionally, for subjects participating in the PK substudy, blood Phe and Tyr samples will be collected at all timepoints from the PK samples, prior to plasma harvesting in Part 1. After the blood samples are withdrawn, the blood will be applied to the VAMS device for Phe and Tyr collection and analysis (ie, no additional finger prick will be required for collection of these samples). Sepiapterin and BH_4 plasma concentrations will be determined by Syneos Health, using a validated liquid chromatography with tandem mass spectrometry method. 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.

Table 19 and Table 20 in Appendix A present sampling times and permitted windows.

8.1.8.1. Part 1

Blood samples (at least 1 mL; duplicate samples should be taken at each timepoint) will be collected from a subset of subjects to characterize the PK of sepiapterin and BH_4.

Approximately 30 subjects aged ≥12 years will participate in the PK substudy. At least 12 out of the 30 subjects of age ≥12 years participating in the PK substudy will be female. In addition, up to 6 subjects from these age groups will be included: 1) <2 years; 2) 2 to 5 years; and 3) 6 to 11 years.

For subjects aged <2 years:

- Part 1 Day 1: Predose and 4 hours postdose
- Part 1 Day 14: 2 and 6 hours postdose

For subjects aged ≥2 years:

- Part 1 Day 1: Predose and 0.5, 1, 2, 4, 6, 8, and 24 hours postdose

8.1.8.2. Part 2

Sparse blood samples (at least 1 mL; duplicate samples should be taken at each timepoint) will be collected from a subset of subjects to characterize the PK of sepiapterin and BH_4. To achieve sparse sampling from 30 subjects in Part 2, this subset of subjects will consist of those who participated in the Part 1 PK substudy and continued into Part 2 and also additional subjects who did not participate in the Part 1 PK substudy. Samples will be collected at the following timepoints:

- Part 2 Days 1, 14, 28, and 42: Predose and 4 hours postdose

8.1.9. *PAH* Genotype Testing

PAH genotype testing will not be required for inclusion/exclusion; however, all subjects will undergo genotyping during screening, unless documented in their medical history, and these data will be collected for analysis.

8.1.10. Quality of Life Baseline Assessments

Testing will be performed at Part 2 Day 1 (predose). The following tests will be performed:

- PKU-QOL: Parent PKU-QOL (6 to 8 years), Child PKU-QOL (9 to 11 years), Adolescent PKU-QOL (12 to 17 years), and Adult PKU-QOL (≥18 years) to 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)

8.1.10.1. Phenylketonuria Quality of Life Questionnaire

In this study, a PKU-specific version of the quality of life (QOL) questionnaire will be used. Four versions of the PKU-QOL will be used: parent (ages 6 to 8 years old, 54 items), child (9 to 11 years old, 40 items), adolescent (12 to 17 years old, 58 items), and adult (≥18 years, 65 items). Each version is available 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.

Phenylketonuria quality of life questionnaires 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 focused on the past 1 week for all sections except for 'patient's general feeling' where the recall period was 'in general'. 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).

8.1.10.2. European Quality of Life - 5 Dimensions

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).

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 subject 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.

8.2. Adverse Events and Serious Adverse Events

8.2.1. Definition of Adverse Events

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

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

- All AEs during the course of treatment with study drug administration
- All AEs resulting from medication misuse, abuse, withdrawal, or overdose, of study drug
- All AEs resulting from medication errors, such as dispensing or administration error outside of what is described in the protocol
- Apparent unrelated illnesses, including worsening of a pre-existing illness

- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate AEs. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents.
- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test)
- Laboratory abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event should be captured in the source documents. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall laboratory monitoring and should not be reported as AEs.
- Pre-existing condition (eg, allergic rhinitis) must be noted on the appropriate eCRF for screening but should not be reported as an AE unless the condition worsens or the episodes increase in frequency during the AE reporting period. Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that occurs during the treatment with study drug should be reported as the AE, and the resulting appendectomy should be recorded in the source documents and eCRF. If a surgical procedure was planned prior to entry into the study, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an AE. Note that, as described in Section 8.2.2, any hospitalization occurring as the consequence of an AE during the study period should be reported as an SAE.

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

8.2.2. Definition of Serious Adverse Events

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

- Result in death. This includes all deaths on treatment or within 30 days after last study drug administration, including deaths due to disease progression. Any death occurring later than 30 days following the last dose need not be reported as an SAE unless it is a result of an event that started within the period covered by the on-study definition. The reported AE should be the event that caused the death. In addition, any AE resulting in death that occurs subsequently to the AE reporting period and that the investigator assesses as possibly related to the study drug should also be reported as serious.

- Is life-threatening. This refers to an event in which the subject was at risk of death at the time of the event. It does not include an event that, had it occurred in a more severe form, hypothetically might have caused death.
- Requires hospitalization or prolongation of existing hospitalization (excluding hospitalizations for administration of the study drug, procedures required by the study protocol, or treatment-related diagnostic procedures; other planned hospitalizations; or hospitalizations related only to progression of disease). Emergency room visits that do not require admission to the hospital do not fall into this category, but the event may be serious due to another seriousness criterion.
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions.
- Any other medically important event that the investigator or the sponsor judges to be serious or which is defined as serious by the regulatory agency in the local country. These are AEs that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Medical judgment should be exercised in deciding whether an AE is serious based on above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.
- A pregnancy resulting in spontaneous abortion, stillbirth, neonatal death, or congenital anomaly (including that in an aborted fetus).

All SAEs that occur after any subject has been enrolled, before treatment, during treatment, or within 30 calendar days following the cessation of treatment, whether or not they are related to the study, must be recorded on forms provided by PTC.

8.2.3. Unexpected Adverse Events

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

8.2.4. Eliciting Adverse Event Information

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

8.2.5. Recording Non-Serious Adverse Events and Serious Adverse Events

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

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

- Indication of whether the event is serious or non-serious (Section 8.2.2)
- Relationship to study drug (Section 8.2.6)
- Severity of the event (Section 8.2.7)
- Onset date
- Resolution date, or date of death
- Action taken
- Outcome of the event

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

8.2.6. Describing Adverse Event Relationship to Study Drug

The investigator should provide an assessment of the relationship of the AE to the study drug, ie, whether there is a reasonable possibility that the study drug caused the AE, using the considerations outlined in Table 14.

Table 14: Relationship of Study Drug to AE Relationship

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

Relationship	Description
Unrelated	A clinical event in which a relationship to the study drug seems improbable because of factors such as inconsistency with known effects of the study drug, lack of a temporal association with study drug administration, lack of association of the event with study drug withdrawal or re-challenge, and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the AE to a concomitant drug, medical history of a similar event, the subject's disease state, other medical conditions, or environmental factors.

8.2.7. Grading of Severity of Adverse Events

The severity of AE will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (refer to the study manual). For each episode, the highest severity grade attained should be reported.

If a CTCAE criterion does not exist, the investigator should use the following grade or adjectives to describe the maximum intensity of the AE: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal). For purposes of consistency with the CTCAE, these intensity grades are defined in Table 15.

Table 15: Grading of Adverse Event Severity Grade

Grade	Adjective	Description
Grade 1	Mild	Sign or symptom is present, but it is easily tolerated, is not expected to have a clinically significant effect on the subject's overall health and well-being, does not interfere with the subject's usual function, and is not likely to require medical attention
Grade 2	Moderate	Sign or symptom causes interference with usual activity or affects clinical status and may require medical intervention
Grade 3	Severe	Sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up
Grade 4	Life-threatening	Sign or symptom results in a potential threat to life
Grade 5	Fatal	Sign or symptom results in death

8.2.8. Adverse Event Reporting

Investigator site reporting requirements for AEs are summarized in Table 16.

Table 16: Investigator Site Requirements for Reporting Adverse Events

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

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

Adverse events spontaneously reported by the subject and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. Clinically significant changes in laboratory values, blood pressure, and pulse need not be reported as AEs. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study drug must be reported and recorded as an AE.

Information about AEs and SAEs will be collected from the date of signing of the Informed Consent Form (ICF) until 30 calendar days following the last dose of study drug.

The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), outcome, intensity, causality, action taken with study drug, serious criteria (if applicable), and whether or not it caused the subject to discontinue the study.

Intensity will be assessed according to the scaling demonstrated in Table 15 (Section 8.2.7).

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity (Section 8.2.7), whereas seriousness is defined by the criteria under Section 8.2.2. An AE of severe intensity may not be considered serious.

Should a pregnancy occur in a subject, or a female partner of a subject, it must be recorded on PTC's Pregnancy Form and reported to PTC within 24 hours of first awareness (see contact details below). Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

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

The first day of AE reporting will coincide with the date of signing of informed consent and/or assent (as appropriate).

8.2.9. Serious Adverse Event Reporting

All SAEs should be reported via the "SAE Report Form" to PTC immediately, without undue delay, but under no circumstances later than 24 hours of becoming aware of the event(s). In addition, the AE portion of the eCRF must also be completed in EDC.

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

Follow-up information to the SAE should be clearly documented with "Follow-up" box checked and the follow-up number in the SAE Report Form completed and faxed or e-mailed to the same party. All follow-up SAE Report Forms for the event must be signed by the investigator. Any source documents (eg, progress notes, nurses' notes, laboratory and diagnostic test results, and discharge summaries) provided to the sponsor should be redacted so that the subject's name, address, and other personal identity information are obscured. Only the subject's study number and initials are to be provided (in regions where the provision of such information is permitted). The information in the AE portion of the eCRF and the SAE Report Form(s) must match or be reconciled. Where the same data are collected, the information on the SAE Report Form must be completed in a consistent manner.

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

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

PTC Safety Department
Attention: Pharmacovigilance
E-mail: [redacted], Facsimile: [redacted]

PTC is responsible for notifying the relevant regulatory authorities of certain events. It is the PI's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

8.2.10. Contraception

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

Women of childbearing potential must use at least 1 form of highly effective contraception for the duration of study participation and for 90 days after last administration of study drug in a manner such that risk of failure is minimized. Periodic and/or temporary abstinence such as declaration of abstinence during study participation or fertility awareness-based methods to prevent pregnancy (including but not limited to symptothermal and ovulation estimation by either calendar day or salivary/cervical secretions) are not considered effective methods of birth control; however, true (absolute) sexual abstinence (ie, in line with the preferred and usual lifestyle of the subject) may be permitted. Highly effective methods of birth control approved for use in this study are as follows:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Intravaginal
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral
  - Injectable
  - Implantable
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Vasectomized partner with confirmed azoospermia

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

8.2.11. Reporting Pregnancy

PTC should be notified in the event that a female subject in the study, or a female partner of a male subject in the study, becomes pregnant on-study or within 90 days of the last administration of study drug; this must be reported on a Pregnancy Notification Form (see Study Manual for details). This must be done whether or not an AE has occurred and within 24 hours of awareness of the pregnancy. The information submitted should include the anticipated date of birth or pregnancy termination.

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

In any of the four situations listed below, the subject will be provided with a "Pregnancy/Pregnant Partner Data Release Form" to request their consent to follow the progress of the pregnancy and the birth and the health of their child.

- Subject becomes pregnant while participating in the study

- Female partner of a male subject participating in the study becomes pregnant
- Subject becomes pregnant up to 90 days after the last administration of the study drug (60 days after the 30-day safety follow-up telephone call)
- Female partner of a male subject participating in the study becomes pregnant up to 90 days after the partner completed the study

Because the risk to an unborn child is unknown, the subject should be asked to sign this consent form. However, signing this form is voluntary; it is up to the subjects to decide whether to agree to the collection of this information or not. Upon signing, the subject's and the child's medical records relating to the pregnancy and delivery, and the health of the child, will be reviewed for up to 1 year of age.

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

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

All information collected with regard to the pregnancy, the delivery of the child, and the health of the child is confidential to the limit allowed by law. These data will be coded to hide the subject's identity and the identity of the child. In particular, the subject's name and the child's name will not be reproduced on any other paper or electronic document. These data will not be disclosed voluntarily by PTC. However, regulatory agencies may have to examine these data to ensure that the study is done properly.

Of note, if the pregnant female partner of a male subject participating in the study does not wish to provide such information, this will not prevent the partner from continuing with the study.

8.3. PTC Adverse Event Reporting Requirement to Regulatory Authorities, Investigators, and IRB/IEC

As the sponsor of the study, PTC is responsible for reporting any suspected unexpected serious adverse reactions, and any other applicable SAEs, to regulatory authorities, IRB/IEC, investigators, and study sites in an expedited manner, in accordance with national regulations in the country. The initial expedited safety report will be provided as required according to local regulations (eg, within 15 days) after the earliest date PTC or an agent of PTC (eg, a site monitor) becomes aware of a SAE. This awareness date is the date the regulatory reporting clock begins, and the date is considered Day 0.

The investigator should also forward a copy of all expedited reports to IRB/IEC, as per local requirements. SAEs will not be reported to the IRB/IEC immediately, but instead will be sent periodically via inclusion in the annual safety report (eg, Development Safety Update Report) to the reviewing committee per local requirements.

9. STATISTICAL CONSIDERATIONS

A statistical analysis plan (SAP) will be prepared and approved prior to study unblinding to provide a more detailed description of the nature of the analyses and the manner in which results will be compiled. The SAP will include details of the statistical models to be used and the test statistics to be employed.

9.1. Statistical Hypotheses

The null hypothesis of this study is that the mean change in Phe levels from baseline to end of the double-blind treatment period is the same in the PTC923 arm and in the placebo arm versus the alternative that they are different; a 2-sided test at the 5% alpha level will be applied.

9.2. Sample Size Determination

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 ≥2 years of age 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.

9.3. Population for Analyses

Four study populations will be analyzed:

- 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.

- Per Protocol (PP) Analysis Set: The 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. The PP Analysis Set will be used for sensitivity analysis of the primary efficacy endpoint. The criteria for inclusion in the PP Analysis Set will be finalized prior to study unblinding and detailed in the SAP.
- 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. Subjects will be analyzed according to actual treatment received.
- 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.

9.4. Statistical Analyses

9.4.1. General Approach

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

9.4.2. Analysis of Efficacy Endpoints

All efficacy analyses will be performed based on the FAS. The primary endpoint analysis will also be conducted using the PP Analysis Set.

For the primary efficacy endpoint, a gatekeeping procedure will be used to control the family-wise error rate:

The stratum of subjects ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 will be tested at the significance level of 0.05 (2-sided). If $p<0.05$, then the study will be declared positive in this stratum.

Only if the stratum of subjects ≥2 years of age with mean percent reduction in Phe levels of ≥30% during Part 1 is statistically significant at the 0.05 level, will the overall study with all subjects ≥2 years of age with a mean percent reduction in Phe levels of ≥15% during Part 1 be tested, also at the 0.05 significance level.

For the primary endpoint of reduction in blood Phe for the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1, a mixed model repeated measures (MMRM) model will be fitted using the available blood Phe data. The response variable is the change from baseline in blood Phe measured at postbaseline assessments for each subject. The model will include fixed effects for treatment, baseline blood Phe, baseline Phe stratum (<600 μmol/L or ≥600 μmol/L), visit (categorical), 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 for the average change at Part 2 Weeks 5 and 6 will be used to perform treatment group comparisons. The LS means, treatment effect estimate, confidence interval, and 2-sided p value will be presented. Corresponding LS means from the same MMRM model will be used to estimate the treatment effects at each visit week.

For the primary endpoint of reduction in blood Phe levels for all subjects, the same MMRM model used for the stratum of subjects with mean percent reduction in blood Phe levels of ≥30% during Part 1 will be used with the addition of a fixed effect for Phe reduction randomization strata (≥15% or ≥30%).

The secondary efficacy endpoint of 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 an MMRM model. 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 will be estimated and compared between treatment arms.

9.4.3. Missing Data

For the primary analysis of the primary endpoint, MMRM model will be used on the available data assuming the missing assessments are missing at random (MAR). Missing data will be imputed using the multiple imputation procedure (Rubin 1987, Ouyang 2017) to further assess the influence of missing primary endpoint data and details of the method will be provided in the SAP. Briefly, these methodologies will include multiple imputation under an assumption of missing not at random and tipping-point analyses to explore how extreme the difference between randomized treatments would have to be among subjects with missing data to overwhelm the treatment effect obtained in the primary. In these analyses, the multiple imputation approach will be expanded to allow for varying impact of missing data by incorporating a shift parameter in the imputation model. The range of values for the shift parameter will explore the varying missing data assumptions, including the scenario where the imputation model is completely based on the control group (ie, control-based imputation).

9.4.4. Pharmacokinetic Analyses

Pharmacokinetic parameters and plasma concentrations will be summarized separately following different PK sampling scheme and population using descriptive statistics.

Additionally, depending on the number of samples collected and subject demography, a population PK analysis will be performed using all PK samples collected in the study to further characterize the PK of sepiapterin and BH_4 to examine potential source of PK variability, such as age, and to explore the relationship between exposure and response.

9.4.5. Safety Analyses

9.4.5.1. Subject Disposition

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

9.4.5.2. Medical History and Prior Medication

Medical history and prior medication information will be descriptively summarized.

9.4.5.3. Extent of Exposure and Treatment Compliance

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

9.4.6. Baseline Descriptive Statistics

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

9.4.6.1. Laboratory Parameters

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

9.4.6.2. Adverse Events

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

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

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

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

10.1. Study Monitoring

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

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

10.2. Audits and Inspections

Authorized representatives of PTC, a regulatory authority, or an IEC/IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a PTC audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The investigator should contact PTC immediately if contacted by a regulatory agency about an inspection.

10.3. Institutional Review Board/Independent Ethics Committee

The investigator (or designee) will submit this protocol, any protocol modifications, and the subject consent/assent form to be utilized in this study, to the appropriate IRB/IEC for review and approval. Documentation of approval of the protocol and the informed consent/assent document must be provided to PTC (or designee) prior to initiation of this study.

The investigator is responsible for assuring continuing review and approval of the clinical study. The investigator must also promptly report all changes in the research activity and all unanticipated problems involving risk to the subjects or others to his/her IRB/IEC. The investigator will not make any changes in the protocol without IRB/IEC approval except as necessary to eliminate apparent immediate hazards to the subjects. The investigator will provide progress reports to the IRB/IEC as required by the IRB/IEC. If the study remains in progress for >1 year, the investigator must obtain annual renewal and re-approval from the IRB/IEC. Documentation of renewal must be submitted to PTC (or designee). The investigator will provide notice to the IRB/IEC of completion of participation in the study.

11. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCP and all applicable regulatory requirements, PTC may conduct a quality assurance audit. Please see Section 10.2 for more details regarding the audit process.

12. ETHICS

12.1. Ethics Review

The final study protocol, including the final version of the ICF, must be approved, or given a favorable opinion in writing by an IRB/IEC as appropriate. The investigator must submit written approval to PTC before he or she can enroll any subject into the study.

The investigator is responsible for informing the IRB/IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB/IEC must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The investigator is also responsible for providing the IRB/IEC with reports of any reportable serious adverse drug reactions from any other study conducted with the study drug. PTC will provide this information to the investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

12.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements, and PTC's policy on Bioethics.

12.3. Informed Consent Process

By signing the protocol, the investigator assures that informed consent or assent (as required) will be obtained from each subject or legally authorized representative prior to study entry and that the informed consent will be obtained in accordance with current regulations.

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

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

13. DATA HANDLING AND RECORDKEEPING

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

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

13.1. Inspection of Records

PTC will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

13.2. Retention of Records

The investigator must maintain all documentation relating to the study for at least 15 years (or longer depending on local requirements) after completion or discontinuation of the trial or at least 2 years after the granting of the last marketing authorization in the EU (when there are no pending or contemplated marketing applications in the EU) or for at least 2 years after formal discontinuation of clinical development of the investigational product, whatever is the longest.

If it becomes necessary for PTC or the regulatory authority to review any documentation relating to the study, the investigator must permit access to such records.

13.3. Protocol Deviations

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

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits (excluding COVID-19 related site closures)
- Drug dosing not administered within the time frame specified in the protocol

- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc. - either tests not done, incorrect tests done, or not done within the time frame specified in the protocol
- Procedural deviations such as incorrect storage of study drug, failure to update the informed consent/assent form when new risks become known, or failure to obtain IRB/IEC approvals for the protocol and informed consent/assent form revisions

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

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

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

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

14. PUBLICATION POLICY

The information developed during the conduct of this clinical study is considered confidential by PTC. This information may be disclosed as deemed necessary by PTC. Clinical trial results will be entered in EudraCT and subsequently will be published on clinicaltrialsregister.eu.

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

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

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

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

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

15. PROTOCOL AMENDMENT HISTORY

Version 1.0: 23 March 2021

Version 2.0: 15 July 2021

Version 3.0: 06 December 2021

Version 4.0: 13January 2022

Version 5.0: 24 June 2022

15.1. Version 5.0: 24 June 2022

The overall reasons for Version 5.0 of the protocol were to incorporate Health Authority feedback and to include additional blood (plasma) sampling.

Protocol Section	Version 5.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis, study schema, and Schedule of Assessments were updated to be consistent with changes in the protocol.	Update
Section 3.1.2.2	Language was added to clarify determination of subject's participation in Part 2 of the study.	Update
Table 1, Section 8.1.5	Table footnote and section were updated to specify confirmatory serum pregnancy test should be performed at the site's local laboratory.	Update
Table 2	Row and applicable footnote (q) were added to detail blood (plasma) samples should be taken at Part 2 Day 1 (predose) and Part 2 Day 42.	Update
Section 4.1[a]	Inclusion criterion 5 was updated for consistency with CTFG 2020 guidance.	Update
Section 5.4 [a]	Section was updated to correct an error.	Update
Table 5	Table was updated to add additional blood samples taken in Part 2.	Update
Table 13	Table was updated to add additional blood samples taken in Part 2.	Update
Section 8.2.10[a]	Contraceptive requirements were clarified for full alignment with Section 4.1.	Update
Section 8.2.11	A clarification was added around the reporting period for pregnancy after completion of the study.	Update
Section 14 [a]	A sentence was added to specify trial results will be entered into EudraCT.	Update

[a] These changes were previously included in country-specific protocols (Version 4.1 [UK] and Version 4.1 [Denmark]).

15.2. Version 4.0: 13 January 2022

The overall reason for Version 4.0 of the protocol was to incorporate Health Authority feedback and to update blood sample volumes.

Protocol Section	Version 4.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis, study schema, and Schedule of Assessments were updated to be consistent with changes in the protocol.	Update

Protocol Section	Version 4.0/Update	Reason/ Rationale
Section 3.1.4	Additional detail was added to clarify enrollment period and provide an end of study definition.	Update
Table 1	Footnote g was added to specify eGFR should be calculated.	Update
Table 2	An additional row and footnote (h) was added to explicitly specify when height should be measured. Footnote was added to specify when eGFR should be calculated. Table note was added to provide details around unscheduled visits and eGFR calculation.	Update
Section 4.2	Exclusion criterion 13 was expanded to include specific pathogenic mutations.	Update
Section 4.4.1	Additional detail was added around discontinuation of pediatric subjects.	Update
Section 5.4	Clarifications of total blood volumes were provided by age group.	Update
Section 8.1.2	Additional detail was added when height should be measured for subjects <18 years of age.	Update
Section 8.1.5	Additional detail around timepoints for eGFR calculations was added.	Update

15.3. Version 3.0: 06 December 2021

The overall reason for Version 3.0 of the protocol was to incorporate feedback from various Health Authorities.

Protocol Section	Version 3.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis, study schema, and Schedule of Assessments were updated to be consistent with changes in the protocol.	Update
Protocol	It was updated that Phe responsiveness is defined as mean Phe reduction is ≥15% for subjects <2 years.	Update
Section 1.7	A Risk/benefit assessment section was added.	Update
Section 3.1.4, Section 3.1.5, Table 2	The definition of End of Study was updated for subjects <2 years of age.	Update
Table 1, Section 7.1	The row for Enrollment was removed. Footnote j was updated to specify that analysis of the screening blood Phe can be performed at the site's local laboratory.	Update
Table 2	A row for Enrollment was added. Footnote o was updated with sampling timepoints for Part 2.	Update
Section 4.1, Section 8.2.11	Criterium 3 was updated to specify "uncontrolled" blood Phe levels while on current therapy. Criterium 5 was updated that effective contraception/abstinence must be utilized for up to 90 days after the last dose of study drug.	Update
Section 4.4.1	Additional detail was added to specify requirements around subjects who discontinue from treatment but not from study.	Update
Section 5.2	Additional detail was added around the timing of dosing on PK sampling days.	Update
Section 5.4	Total blood volume was updated	Update
Section 6.1	Details of the excipients were added.	Update
Section 8.1.2	Specified that vital sign measurement should occur prior to laboratory sampling.	Update
Section 8.1.4	Specified that ECGs reads must be taken at least 1 minute apart.	Update
Section 8.1.6	A section was added to detail assessment of laboratory abnormalities.	Update

Protocol Section	Version 3.0/Update	Reason/ Rationale
Section 8.1.8.2	PK sampling timepoints were updated.	Update
Section 8.2.9	SAE reporting timeframe was updated.	Update
Section 8.2.11	Reporting timeframe was updated.	Update

15.4. Version 2.0: 15 July 2021

The overall reasons for Version 2.0 of the protocol were to include subjects from birth and to provide the appropriate age- and weight-based dosing for these subjects.

Protocol Section	Version 2.0/Update	Reason/ Rationale
Protocol	The version number and date were updated throughout. Editorial and administrative revisions (eg, typographical error, punctuation, tenses, abbreviations) were incorporated to provide clarity. The synopsis, study schema, and schedule of assessments were updated to be consistent with changes in the protocol.	Update
Protocol	The dosing schedule was updated throughout to detail age-dependent dosing. Screening visit was updated to up to 45 days. Window for the ETV was extended to 21 days of last dose Specified throughout that subjects from birth can be included in this study. Statement was removed throughout that all subjects will receive PTC923 60 mg/kg in Part 1 of the study. Statement was added throughout to include specific details on subjects <2 years of age with ≥30% response to PTC923 and enrollment into Study PTC923-MD-004-PKU. Statement was added throughout to state subjects who experience <15% reduction (≥2 years of age) or <30% reduction (<2 years of age) will be classified as nonresponsive and will be contacted to schedule an ETV (on or between Day 28 to Day 35). Specified throughout that the Days -1 and 1 samples for Parts 1 and 2 should be taken predose.	Update
Section 3.1.1, Section 5.3, Section 8.1.1	Specified that specific information pertaining to previous use of sapropterin and pegvaliase-pqpz should be collected.	Clarification
Section 3.1.1.1, Table 12	Specified that blood Phe levels should be measured on Days 1, 20, and 30 during the Pegvaliase-pqpz washout.	Update
Section 3.1.2.1, Table 2, Section 8.1.8	Additional details added regarding collection of blood Phe and Tyr samples and timepoints for subjects participating in the PK substudy.	Update
Section 3.1.2.2	Specified that blood Phe levels should be measured on Days 19, 24, and 28.	Update
Table 1, Section 3.1.2.2	Randomization at screening row was removed. Table was updated to include blood Phe/Tyr sample collection at Screening Footnote j was updated to specify that blood Phe/Tyr samples collected at home will be shipped to the study site.	Updates

Protocol Section	Version 2.0/Update	Reason/ Rationale
Table 2	Updated to detail age dependent dosing during Part 1 open-label treatment. Day 15 was removed. Randomization was moved to occur on Part 2 Day -1 Pregnancy testing was removed from Part 1 Days 2 and 15 and Part 2 Days 14 and 28. Footnote c was updated to specify a ±2-day window for Part 2 Day 42 visit. Footnote m was updated to specify sampling windows for blood Phe/Tyr sampling at home. Footnote q was updated to detail study drug return procedure.	Updates
Section 4	"Biochemically diagnosed" was added classical PKU	Clarification
Section 4.1	Criteria 5 and 6 were updated to align with current (2020) CTFG guidance. Criterion 14 clarified surgery was not permitted within 90 days of screening	Update
Section 4.2	Criteria 10 and 11 were added to exclude subjects with renal impairment/disease.	Update
Table 4	Total blood volume for Part 1 was updated.	Update
Table 5	Total blood volume for Part 2 was updated.	Update
Section 5.5	Instructions for returning used/unused drug were updated	Update
Section 5.6, Section 5.6.1	Statement added regarding unblinding and continuing study participation.	Update
Section 6.5	Age-based dosing specifications were updated and added.	Update
Table 12	DBS study day and pegvaliase-pqpz sample days updated.	Update
Table 13	Part 1 and 2 PK sampling time windows updated	Update
Table 13	Specified that the PTC923 washout should be a minimum of 14 days. Day 15 was removed. Specified that the additional sample taken at the ETV should be a DBS.	Update
Section 7.1	Updated to include sample collection on Day -1 and Day 1 for both Part 1 and Part 2.	
Section 8.2.1	Language was updated as per PTC standard procedures.	Update
Section 8.2.8	Language was updated as per PTC standard procedures.	Update
Section 8.2.9	Language was updated as per PTC standard procedures.	Update
Section 8.2.11	Language was updated as per PTC standard procedures.	Update
Section 8.3	Section was added as per PTC standard procedures.	Update

16. REFERENCES

Al Hafid, N and Christodoulou, J. Phenylketonuria: A Review of Current and Future Treatments. Transl Pediatr 2015;4(4):304-317.

Alptekin, IM, Koc, N, Gunduz, M and Cakiroglu, FP. The Impact of Phenylketonuria on PKU Patients' Quality of Life: Using of the Phenylketonuria-Quality of Life (PKU-QOL) Questionnaires. Clin Nutr ESPEN 2018;27:79-85.

BioMarin (2020). Palynziq Prescribing Information.

Bratkovic, D, Margvelashvili, L, Tchan, MC, Nisbet, J, Smith, N. PTC923 (Sepiapterin) Lowers Elevated Blood Phenylalanine in Subjects with Phenylketonuria: A Phase 2 Randomized, Multi-center, Three-period Crossover, Open-label, Active Controlled, All-comers Study. Metabolism 2022;128.

Burnett, JR. Sapropterin Dihydrochloride (Kuvan/Phenoptin), an Orally Active Synthetic Form of BH_4 for the Treatment of Phenylketonuria. IDrugs 2007;10(11):805-813.

Camp, KM, Parisi, MA, Acosta, PB, Berry, GT, Bilder, DA, Blau, N, et al. Phenylketonuria Scientific Review Conference: State of the Science and Future Research Needs. Mol Genet Metab 2014;112(2):87-122.

CDC. (2010a). "WHO Data Table for Boys Length-for-Age and Weight-for-Age Charts (0 to 24 months)." from https://www.cdc.gov/growthcharts/who/boys_length_weight.htm.

CDC. (2010b). "WHO Data Table for Girls Length-for-Age and Weight-for-Age Charts (0 to 24 months)." from https://www.cdc.gov/growthcharts/who/girls_length_weight.htm.

CTFG. (2020). "Clinical Trial Facilitation Group: Recommendations Eelated to Contraception and Pregnancy Testing in Clinical Trials." Retrieved 08 March 2021, from https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf.

European Parliament (2008). Ethical Considerations for Clinical Trials on Medicinal Products Conducted with the Paediatric Population: Recommendations of the Ad Hoc Group for the Development of Implementing Guidelines for Directive 2001/20/EC Relating to Good Clinical Practice in the Conduct of Clinical Trials on Medicinal Products for Human Use.

Fisch, RO. Comments on Diet and Compliance in Phenylketonuria. Eur J Pediatr 2000;159 Suppl 2:S142-144.

Harding, CO, Amato, RS, Stuy, M, Longo, N, K., BB and Posner, J. Pegvaliase for the Treatment of Phenylketonuria: A Pivotal, Double-blind Randomized Discontinuation Phase 3 Clinical Trial. Mol Genet Metab 2018;124:20-26.

Hillert, A, Anikster, Y, Belanger-Quintana, A, Burlina, A, Burton, BK, Carducci, C, et al. The Genetic Landscape and Epidemiology of Phenylketonuria. Am J Hum Genet 2020;107(2):234-250.

Ichiyama, A, Nakamura, S, Nishizuka, Y and Hayaishi, O. Enzymic Studies on the Biosynthesis of Serotonin in Mammalian Brain. J Biol Chem 1970;245(7):1699-1709.

Jurecki, ER, Cederbaum, S, Kopesky, J, Perry, K, Rohr, F, Sanchez-Valle, A, et al. Adherence to Clinic Recommendations Among Patients with Phenylketonuria in the United States. Mol Genet Metab 2017;120(3):190-197.

Kaufman, S. An Evaluation of the Possible Neurotoxicity of Metabolites of Phenylalanine. J Pediatr 1989;114(5):895-900.

KUVAN USPI. (2020). "KUVAN United States Prescribing Information." Retrieved 26 August 2020, 2020, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/022181s020,205065s006lbl.pdf.

Kwon, NS, Nathan, CF and Stuehr, DJ. Reduced Biopterin as a Cofactor in the Generation of Nitrogen Oxides by Murine Macrophages. J Biol Chem 1989;264(34):20496-20501.

Levy, H, Burton, B, Cederbaum, S and Scriver, C. Recommendations for Evaluation of Responsiveness to Tetrahydrobiopterin (BH_4) in Phenylketonuria and Its Use in Treatment. Mol Genet Metab 2007;92(4):287-291.

Lovenberg, W, Jequier, E and Sjoerdsma, A. Tryptophan Hydroxylation: Measurement in Pineal Gland, Brainstem, and Carcinoid Tumor. Science 1967;155(3759):217-219.

MacDonald, A, van Rijn, M, Feillet, F, Lund, AM, Bernstein, L, Bosch, AM, et al. Adherence Issues in Inherited Metabolic Disorders Treated by Low Natural Protein Diets. Ann Nutr Metab 2012;61(4):289-295.

Mayer, B, John, M, Heinzel, B, Werner, ER, Wachter, H, Schultz, G, et al. Brain Nitric Oxide Synthase is a Biopterin- and Flavin-containing Multi-functional Oxido-reductase. FEBS Lett 1991;288(1-2):187-191.

Mayer, B and Werner, ER. In Search of a Function for Tetrahydrobiopterin in the Biosynthesis of Nitric Oxide. Naunyn Schmiedebergs Arch Pharmacol 1995;351(5):453-463.

Nagatsu, T, Levitt, M and Udenfriend, S. Tyrosine Hydroxylase. The initial Step in Norepinephrine Biosynthesis. J Biol Chem 1964;239:2910-2917.

Ouyang, J, Carroll, KJ, Koch, G and Li, J. Coping with Missing Data in Phase III Pivotal Registration Trials: Tolvaptan in Subjects with Kidney Disease, a Case Study. Pharm Stat 2017;16(4):250-266.

Rubin, DB (1987). Multiple Imputation for Nonresponse in Surveys; John Wily & Sons, Inc.

Scriver, C and Kaufman, S (2001). Hyperphenylalaninemia: Phenylalanine Hydroxylase Deficiency. In The Metabolic and Molecular Basis of Inherited Disease. C. Scriver, A. Beaudet, W. Sly and R. Childs; New York, USA, McGraw-Hill Inc**:**1667-1724.

Smith, N, Longo, N, Levert, K, Hyland, K and Blau, N. Exploratory Study of the Effect of One Week of Orally Administered CNSA-001 (Sepiapterin) on CNS levels of Tetrahydrobiopterin, Dihydrobiopterin and Monoamine Neurotransmitter Metabolites in Healthy Volunteers. Mol Genet Metab Rep 2019a;21:100500.

Smith, N, Longo, N, Levert, K, Hyland, K and Blau, N. Phase I Clinical Evaluation of CNSA-001 (sepiapterin), a Novel Pharmacological Treatment for Phenylketonuria and Tetrahydrobiopterin Deficiencies, in Healthy Volunteers. Mol Genet Metab 2019b;126(4):406-412.

Thomas, J, Levy, H, Amato, S, Vockley, J, Zori, R and Doimmock, D. Pegvaliase for the Treatment of Phenylketonuria: Results of a Long-term Phase 3 Clinical Trial Program (PRISM). Mol Genet Metab. 2018;124:27-38.

Tietz, A, Lindberg, M and Kennedy, EP. A New Pteridine-requiring Enzyme System for the Oxidation of Glyceryl Ethers. J Biol Chem 1964;239:4081-4090.

van Wegberg, AMJ, MacDonald, A, Ahring, K, Bélanger-Quintana, A, Blau, N, Bosch, AM, et al. The Complete European Guidelines on Phenylketonuria: Diagnosis and Treatment. Orphanet J Rare Dis 2017;12(1):162.

Vockley, J, Andersson, HC, Antshel, KM, Braverman, NE, Burton, BK, Frazier, DM, et al. Phenylalanine Hydroxylase Deficiency: Diagnosis and Management Guideline. Genet Med 2014;16(2):188-200.

Waisbren, SE, Noel, K, Fahrbach, K, Cella, C, Frame, D, Dorenbaum, A, et al. Phenylalanine Blood Levels and Clinical Outcomes in Phenylketonuria: A Systematic Literature Review and Meta-analysis. Mol Genet Metab 2007;92(1-2):63-70.

Walter, JH, White, FJ, Hall, SK, MacDonald, A, Rylance, G, Boneh, A, et al. How Practical are Recommendations for Dietary Control in Phenylketonuria? Lancet 2002;360(9326):55-57.

17. APPENDICES

APPENDIX A. SAMPLING TIMEPOINTS AND PERMITTED WINDOWS

Table 17: 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 min

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 18: Electrocardiogram 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
≤4 hours after study drug administration		±15 min
>4 hours and ≤24 hours after study drug administration		±30 min

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 19: 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 20: 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

Signature Page for Global PTC923-MD-003-PKU Clinical Protocol V5.0 - 24JUN2022 v

Clinical Approval	[redacted] I approve the document(s) 24-Jun-2022 16:33:40 GMT+0000

Clinical Approval	[redacted] I approve the document(s) 24-Jun-2022 18:19:58 GMT+0000

Statistics Approval	[redacted] I approve the document(s) 27-Jun-2022 05:24:39 GMT+0000

Signature Page for VV-CLIN-004648 v7.0