# CLINICAL STUDY PROTOCOL

| | |
|---|---|
| **Protocol Number:** | PTC923-PKU-301 |
| **Protocol Title:** | A Phase 3, Randomized, Crossover, Open-Label, Active-Controlled Study of Sepiapterin Versus Sapropterin in Participants With Phenylketonuria ≥2 Years of Age |
| **Name of Study Intervention:** | Sepiapterin |
| **Study Phase:** | 3 |
| **Approval Date:** | V4.0 06 September 2024 |
| **IND Number:** | Not applicable |
| **CTIS ID Number:** | 2023-506238-61-00 |
| **Sponsor:** | PTC Therapeutics, Inc.<br>500 Warren Corporate Center Drive<br>Warren, New Jersey 07059<br>USA |

## PTC THERAPEUTICS PROTOCOL APPROVAL SIGNATURES

[redacted]
PTC Therapeutics, Inc.

**Date**

[redacted]
PTC Therapeutics, Inc.

**Date**

[redacted]
PTC Therapeutics, Inc.

**Date**

The eSignature page is located on the last page.

## 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: _____

## 1. SYNOPSIS

<table>
<tr><td colspan="3">Name of Sponsor/Company:<br>PTC Therapeutics, Inc.</td></tr>
<tr><td colspan="3">Name of Study Intervention:<br>Sepiapterin</td></tr>
<tr><td>Protocol Number: PTC923-PKU-301</td><td>Phase: 3</td><td>Countries: Australia, Canada, Czech Republic, Denmark, France, Georgia, Germany, Italy, Netherlands, Poland, Slovenia, Spain, United Kingdom</td></tr>
<tr><td colspan="3">Title of Study: A Phase 3, Randomized, Crossover, Open-Label, Active-Controlled Study of Sepiapterin Versus Sapropterin in Participants With Phenylketonuria ≥2 Years of Age</td></tr>
<tr><td colspan="3">Study Rationale:<br>To evaluate the efficacy and demonstrate significant benefit of sepiapterin compared with the approved dose of sapropterin in reducing blood phenylalanine (Phe) levels in participants with phenylketonuria (PKU).</td></tr>
<tr><td colspan="2">Objectives:</td><td>Endpoints:</td></tr>
<tr><td colspan="3">Primary:</td></tr>
<tr><td colspan="2">• To compare the efficacy of sepiapterin to sapropterin in reducing blood Phe levels in participants with PKU</td><td>• Mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period (the average of the last 2 weeks of each treatment period) in Part 2</td></tr>
<tr><td colspan="3">Secondary:</td></tr>
<tr><td colspan="2">• To evaluate the efficacy of sepiapterin in reducing blood Phe levels<br>• To assess the safety and tolerability of sepiapterin</td><td>• Proportion of participants with baseline blood Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L after each treatment period in Part 2<br>• Proportion of participants reaching blood Phe <360 µmol/L after each treatment period in Part 2<br>• AEs, physical examinations, vital sign assessments, 12-lead ECGs, and routine clinical laboratory assessments</td></tr>
<tr><td colspan="3">Exploratory:</td></tr>
<tr><td colspan="2">• To evaluate changes in blood Tyr over time, including the Phe:Tyr ratio<br>• To assess the taste, palatability, and acceptability (<18 years) of sepiapterin<br>• To evaluate sepiapterin effect on QOL using the PKU-QOL questionnaire in the subset of participants who are able</td><td>• Changes in blood Tyr over time, including the Phe:Tyr ratio<br>• Taste, palatability, and acceptability scores (participants <18 years)<br>• Changes from baseline in QOL using PKU-QOL questionnaire in the subset of participants who are able to complete the PKU-QOL (ie,</td></tr>
</table>

| | |
|---|---|
| to complete the PKU-QOL (ie, participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years, Parent PKU-QOL; ages 9 to 11 years, Child PKU-QOL; ages 12 to 17 years, Adolescent PKU-QOL; and ages ≥18 years, Adult PKU-QOL)<br>• To evaluate sepiapterin effect of QOL using the EQ-5D (EQ-5D-Y Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and EQ-5D-5L ([≥16 years]) | participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years, Parent PKU-QOL; ages 9 to 11 years, Child PKU-QOL; ages 12 to 17 years, Adolescent PKU-QOL; and ages ≥18 years, Adult PKU-QOL)<br>• Changes from baseline in QOL using the 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]) |

**Abbreviations**: AE, adverse event; ECG, electrocardiogram; 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; Phe, phenylalanine; PKU, phenylketonuria; PKU-QOL, Phenylketonuria-Quality of Life; QOL, quality of life; Tyr, tyrosine

**Study Design:**

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

During this study, participants should continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or dietary Phe consumption) as measured by 3-day diet record collection (weekly during Screening and Part 1 Treatment Period; biweekly during Part 1 Washout Period and Part 2 Treatment and Washout Periods). The diet should remain unchanged throughout the study, regardless of treatment or washout periods.

**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), participants will undergo in-clinic screening procedures to determine study eligibility. Participant demographics and medical history information will be collected. Additionally, specific information related to previous use of sapropterin or pegvaliase-pqpz will be collected (ie, details on duration; dose; if discontinued, the reason(s) why; and if a participant is known to be tetrahydrobiopterin [$BH_4$] responsive). Vital signs, weight, and height measurements will be performed. Phenylalanine hydroxylase (*PAH*) gene genotyping should be performed unless already documented in the participant's medical history. Blood Phe/tyrosine (Tyr) levels will be measured. A full physical examination will be performed, and urine 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). Participants will be instructed to continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) throughout the entire study. Participants will maintain a 3-day diet record during each week of Screening, and a dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for

participants to maintain their usual diet without modification. Participants who enter the study and are receiving $BH_4$ supplementation (eg, sapropterin, KUVAN) at the Screening Visit must complete a 7-day washout period prior to dosing.

**Dietary Control Observation Period (24 to 30 Days):**

Participants will continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) and will maintain a weekly 3-day diet record for 4 consecutive weeks during the Dietary Control Observation Period. Participants with a >20% variance in dietary Phe consumption during the 24- to 30-day Dietary Control Observation Period will be considered screen failures.

**$BH_4$ Supplementation Washout (7 Days):**

Following the initial Screening Visit, eligible participants who are taking $BH_4$ supplementation must discontinue the medication as concomitant treatment/supplementation with $BH_4$ will not be permitted during the study. Participants will continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) and will maintain a 3-day diet record during the $BH_4$ Supplementation Washout. A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants 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 (14 Days):**

Following the initial screening and $BH_4$ Supplementation Washout (as necessary), all eligible participants will be enrolled into Part 1 of the study to test for responsiveness to sepiapterin. Participants will receive sepiapterin 60 mg/kg for 14 days.

On Part 1 Day 1, participants will take their dose of sepiapterin while in the clinic; for all other dosing days, participants will take their dose as an outpatient and prior to coming to the clinic when visits are scheduled. All visits will be in-clinic visits or virtual visits (unless specified as a mandatory in-clinic visit). Study visits will occur on Part 1 Day 1 and Part 1 Day 14. On Day 1, palatability and acceptability assessments will be performed for all participants <18 years of age. Palatability will be indirectly assessed by the parent(s)/caregiver(s) of participants <5 years of age; taste and palatability will be assessed separately by participants ≥5 to <18 years; acceptability will be assessed by parent(s)/caregiver(s) for all children <12 years of age.

Blood Phe levels will be measured on Days -1 (only 1 sample is required if this is on the same day as $BH_4$ Washout Period Day 7 and the $BH_4$ Washout Period Day 7 sample would not be collected under these circumstances), 1 (predose), 7, 10, and 14 of Part 1. Baseline blood Phe level will be calculated as the mean of the blood Phe levels from dried blood samples taken on Day -1 and Day 1 (predose). Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint. Participants will continue their usual diet without modification and will maintain 3-day diet records (ie, 1 corresponding to each week of the sepiapterin open-label treatment period).

A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.

**Part 1, Sepiapterin Washout (14 Days Minimum/21 Days Maximum):**

Following completion of 14 days of sepiapterin open-label treatment, participants will begin a

minimum 14-day (21-day maximum) washout. No in-clinic study visits are required during the sepiapterin washout. In Part 1, blood Phe levels will be measured on Days 19, 24, and 28 during the sepiapterin washout. If Part 1 Day 28 is the same day as Part 2 Treatment Period 1 Day -1, only 1 dried blood sample each for Phe/Tyr should be collected (the Part 1 Day 28 samples would not be collected under these circumstances). 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. Participants will continue their usual diet without modification and will maintain a 3-day diet record (ie, 1 corresponding to each 2-week period of the sepiapterin washout). A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.
During the sepiapterin washout, the mean change in blood Phe levels over the 14 days of sepiapterin treatment (mean of Part 1 Days 7, 10, and 14) will be measured and compared against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 [predose]). Determination of a participant's sepiapterin responsiveness may be conducted with ≤2 pretreatment blood Phe levels (but requiring a minimum of 1 pretreatment level) and ≤3 sepiapterin treatment blood Phe levels (but requiring a minimum of 1 sepiapterin treatment level), depending on the available data on or between Part 1 Study Day 28 and Day 35. Participants who experience <20% 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 and Day 35).
Part 1 for all participants ends on Day 35. In no circumstances will a participant be permitted to continue in Part 1 beyond Day 35. If a participant's blood Phe concentrations are not available (minimum requirement of 1 pretreatment level and 1 sepiapterin treatment level) for determination of responsiveness to sepiapterin by Day 35 of Part 1, then that participant 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 the PTC-sponsored open-label long-term study, PTC923-MD-004-PKU.

**Part 2, Randomized, Active-Controlled, Open-Label, Crossover Treatment Period (84 Days):**
On Part 2 Treatment Period (TP)1 Day -1, all eligible participants will be randomized 1:1 to 1 of 2 treatment sequences (60 mg/kg sepiapterin-20 mg/kg sapropterin OR 20 mg/kg sapropterin-60 mg/kg sepiapterin). The randomization will be stratified based on their mean % reduction in blood Phe levels from Part 1 (ie, participants with mean % reduction in Phe levels of ≥20 % to <30% and participants with mean % reduction in Phe levels of ≥30%).
Participants will receive each open-label treatment for 4 weeks (TP1 and TP2). Following completion of each 4-week treatment period, participants will complete a washout (14 days [+3-day window]).
For all days where in-clinic visits are scheduled, participants will take their dose of study drug (sepiapterin or sapropterin) while in clinic. A 14-day washout period (+3-day window) occurs after each treatment period (Washout 1 and Washout 2). All visits will be in-clinic visits or virtual visits(unless specified as a mandatory in-clinic visit). Study visits will occur on TP1 Day 1, TP1 Day 28, TP2 Day 1, and TP2 Day 28 to conduct study evaluations, collect 3-day diet records, and capture any AEs and concomitant medications.

Participants will continue their usual diet without modification and will maintain 3-day diet records (ie, 1 corresponding to each 2-week period of Part 2). A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.

During each treatment period, blood Phe levels will be measured in Part 2 on Days -1, 1 (predose), 7, 10, 14, 19, 24, and 28. Baseline is the mean of Day -1 and Day 1 predose blood Phe levels, and the mean blood Phe levels of every 2-week intervals will be calculated (Days 7, 10, and 14 for Weeks 1 and 2 and Days 19, 24, and 28 for Weeks 3 and 4). 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. If Part 2 Washout Period 1 Day 14 is the same day as Part 2 Treatment Period 2 Day -1, only 1 dried blood sample each for Phe/Tyr should be collected (the Part 2 Washout Period 1 Day 14 samples would not be collected under these circumstances).

On Part 2 Day 1 of TP1 and Day 28 of TP1 and TP2, 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]; and Adult PKU-QOL [≥18 years]) will be conducted using the tool in one of the following validated languages: English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the PKU-QOL will not be required for participants whose primary language is not one of the available validated languages. 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.

**End of Study:**

For all participants who complete Part 2, End of Study (EOS) is defined as their final study visit on completion of the 14-day washout (+3-day window) after TP2. All participants who complete Part 2 will be given the opportunity to continue treatment in the sepiapterin open-label long-term study, PTC923-MD-004-PKU. If a participant elects not to continue into Study PTC923-MD-004-PKU, they should have a follow-up phone call 30 (±3) days after their EOS Visit for collection of AEs.

**Early Termination Visit:**

An ETV will be performed on or between Day 28 and Day 35 for all participants who are determined to be nonresponsive to sepiapterin in Part 1 or within 3 days for any participant who discontinues the study prematurely during Part 1 or Part 2 for any other reason. During the ETV, completed 3-day diet records, AEs, concomitant medications, weight, vital signs, electrocardiograms (ECGs), and blood Phe/Tyr levels will be collected. A physical examination, clinical laboratory tests, and a pregnancy test will be performed. For nonresponsive participants and participants who discontinue prematurely, following the ETV, participants may revert to their prestudy treatment for PKU (at the investigator's and the participant's treating physician's discretion), and a follow-up phone call will occur 30 (±3) days after the last dose of study drug to assess for serious adverse events (SAEs).

**Number of Participants/Study Centers:**
Approximately 100 participants will be screened in Part 1 (Open-Label Responsiveness Test) to achieve 42 participants randomized into Part 2 (Active-Controlled, Open-Label, Crossover Treatment Period).
Approximately 18 study centers will participate in this study.

**Diagnosis and Main Criteria for Inclusion:**
Participants with any PAH mutation are permitted to screen and enroll into the study. However, participants with biochemically diagnosed classical PKU will be capped at 30% of the Safety Analysis Set. Biochemically diagnosed classical PKU includes those with ≥2 historical blood Phe concentrations ≥1200 µmol/L in their medical history. Furthermore, participants with initial newborn screen performed >3 days after birth demonstrating values >1200 µmol/L may have these values excluded from the ≥2 historical blood Phe concentrations requirement. Genotyping will not be required for study eligibility; however, all participants will undergo genotyping unless documented in their medical history, and these data will be collected for analysis.

**Inclusion Criteria**:
Participants are eligible to be included in the study only if all the following criteria apply:

1. Informed consent, and if necessary, assent (with parent/legally designated representative consent)
2. Male or female participants ≥2 years of age
3. Blood Phe level ≥360 µmol/L on current therapy anytime during Screening and blood Phe level ≥360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the participant's medical history (inclusive of the Screening value)
4. Clinical diagnosis of PKU with hyperphenylalaninemia 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, ie, fertile, following menarche and until becoming 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 postprocedure.
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**:
Participants are excluded from the study if any of the following criteria apply:

1. The individual, in the opinion of the investigator, is unwilling or unable to adhere to the requirements of the study. Incapacitated adults are not eligible for participation in this study.
2. Gastrointestinal disease (such as irritable bowel syndrome, inflammatory bowel disease, chronic gastritis, 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 any of the ingredients or excipients of 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 participant's ability to participate in the study or increase the risk of participation for that participant
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/1.73 $m^2$
    In participants ≥18 years of age, the Modification of Diet in Renal Disease Equation should be used to determine GFR.

In participants <18 years, the Bedside Schwartz Equation should be used to determine GFR.

12. Requirement for concomitant treatment with levodopa or 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 guanosine triphosphate (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. Unwillingness to washout from $BH_4$ supplementation (eg, sapropterin, KUVAN).
16. Use of pegvaliase-pqpz (PALYNZIQ) concurrently or within the 60 days prior to Screening
17. Greater than 20% variance in dietary Phe consumption as measured by mandatory weekly 3-day diet record collection for 4 consecutive weeks (Dietary Control Observation Period during Screening).

**Study Intervention, Dosage, and Mode of Administration:**
The test product is sepiapterin powder for oral use. All doses will be administered orally, once a day with food.
**Part 1:** All participants will receive 60 mg/kg/day sepiapterin for 14 days.
**Part 2:** All participants will receive 60 mg/kg/day sepiapterin for 4 weeks and 20 mg/kg/day sapropterin for 4 weeks (order determined by randomization).
Sapropterin is provided as sapropterin 100 mg tablets.

**Study Duration**:
The maximum length of time a participant can be in the study is approximately 173 days.
**Treatment Duration**: 10 weeks

**Statistical Methods:**
**Analysis Sets:**
Primary Analysis Set (PAS): All participants who achieved a ≥30% reduction in blood Phe concentrations in Part 1, are randomized, and take at least 1 dose of study drug in Part 2 will be included in the PAS. Participants will be analyzed according to their randomized treatment.
Full Analysis Set (FAS): All participants who are randomized and take at least 1 dose of study drug in Part 2 will be included in the FAS. Participants 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 participants 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.
Safety Analysis Set: All participants who receive at least 1 dose of study drug, including during Part 1, will be included in the Safety Analysis Set. Participants will be analyzed according to the actual treatment received.
**Efficacy:**
All efficacy analyses will be performed based on the PAS and FAS. The primary endpoint analysis will also be conducted using the PP Analysis Set as a supportive analysis.
For the primary efficacy endpoint, a gatekeeping procedure will be used to control the familywise error rate:

The stratum of participants with mean % reduction in Phe levels of ≥30% during Part 1 (PAS) will be tested at the significance level of 0.05 (2-sided). If $p<0.05$, then the study will be declared positive; otherwise, the study would be declared negative.
Only if the test based on the PAS population is statistically significant at the 0.05 level will the FAS population be tested, also at the 0.05 significance level.

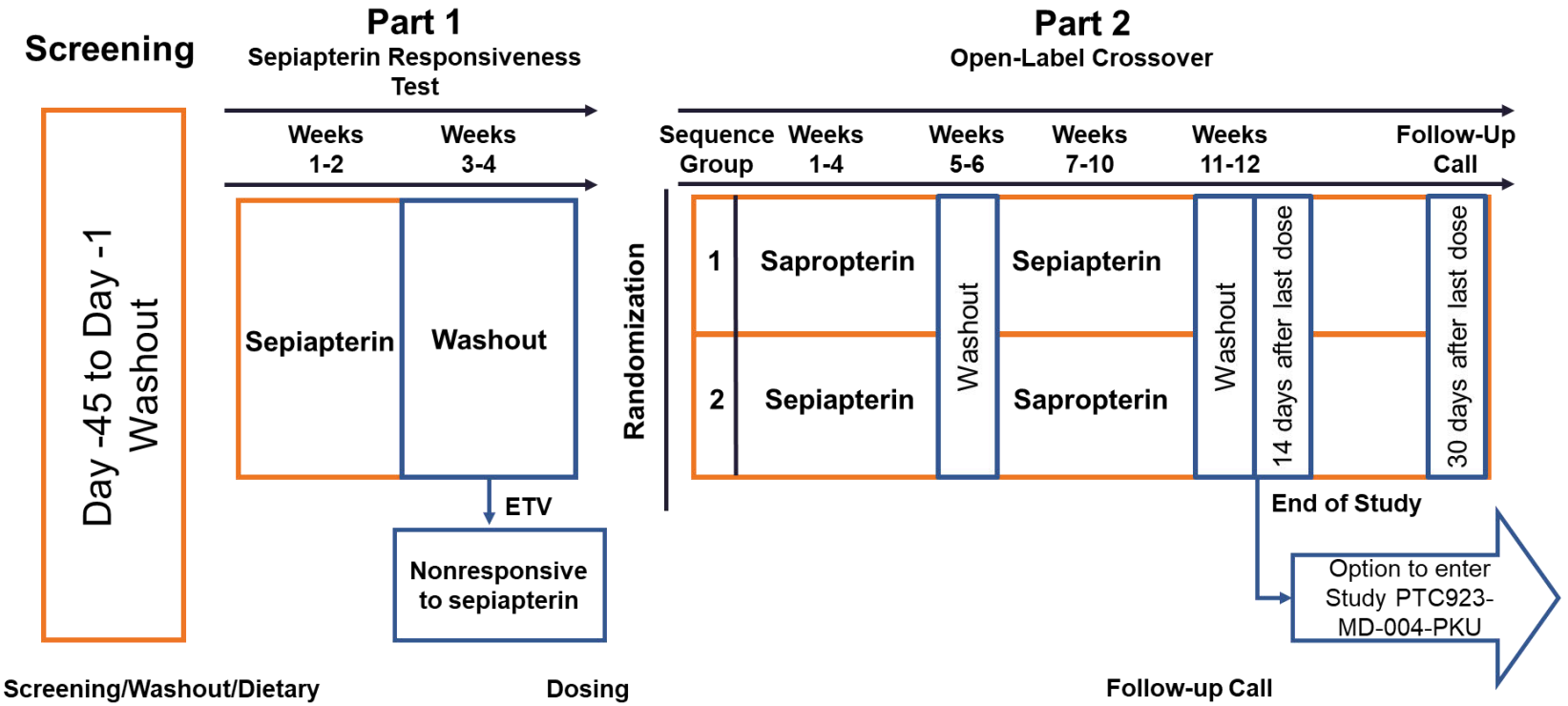
The baseline for Part 1 is the average of Day -1 and Day 1 predose blood Phe concentration values.

For Part 2 Treatment Period 1, the baseline is the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 1. For Part 2 Treatment Period 2, the baseline is the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 2.

A mixed model repeated measures (MMRM) model will be fitted on the calculated mean change in blood Phe from baseline to each of the 2-week postbaseline assessment intervals within the treatment period (Periods 1 and 2) for each participant. The MMRM model will be used on available data assuming the missing assessments are missing at random. The model will include fixed effects for treatment group, sequence, period, % Phe reduction from Part 1 (≥20% to <30% or ≥30%), visit (Weeks 1 to 2 and Weeks 3 to 4), and visit-by-treatment interaction, and the baseline blood Phe (for each period) as a covariate. In addition, participant nested within sequence will be included as a random effect. The least squares (LS) mean estimate for the change in blood Phe levels from baseline to average of Weeks 3 and 4 will be used to perform treatment group comparisons. The LS means, treatment effect estimate, 95% CI, and 2-sided p value will be presented.
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 participant and summarized using descriptive statistics by treatment group.
Palatability:
Palatability, taste, and acceptability evaluation results will be tabulated for each question. Additional summaries will be performed as deemed necessary upon review of the data.
Interim analysis:
No formal interim analysis is planned.

## 1.1. Schema

**Figure 1: Study Schema**




**Screening/Washout/Dietary Observation Period**
Duration determined by Dietary Observation Period (24 to 30 days)[a]

**Dosing**
Sepiapterin 60mg/kg QD
Sapropterin 20mg/kg QD

**Follow-up Call**
Required for participants who are sepiapterin nonresponsive (Part 1), discontinue the study prematurely (ETV), or do not enter into Study PTC923-MD-004-PKU

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

## 1.2. Schedule of Activities

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

| Evaluation | Screening Period (Up to a Total of 45 Days) | | Part 1 - Open-Label Responsiveness Test | | | | | | | | ETV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screening Visit | $BH_4$ Washout Period | Sepiapterin Open-Label Treatment 60 mg/kg | | | | | Sepiapterin Washout | | | |
| | Up to 45 Days | 7 Days[a] | 14 Days | | | | | Min 14 Days Max 21 Days | | | |
| **Study Day** | | | **-1** | **1** | **7** | **10** | **14** | **19** | **24** | **28[b]** | |
| Informed consent/assent | X | | | | | | | | | | |
| PAH genotyping[c] | X | | | | | | | | | | |
| Confirm eligibility | X | | | X | | | | | | X | |
| Demographics | X | | | | | | | | | | |
| Medical history[d] | X | | | | | | | | | | |
| Enrollment | | | | X | | | | | | | |
| In-clinic visit | X | | | X | | | | | | | X |
| Virtual visit | | | | | | | X[e] | | | X[e] | |
| Vital signs,[f] weight | X | | | X[g] | | | | | | | X |
| Height | X | | | | | | | | | | |
| ECGs[h] | | | | X[g] | | | | | | | X |
| Physical examination[i] | X | | | X[g] | | | | | | | X |
| Clinical laboratory tests[j] | X[k] | | | X[g] | | | | | | | X |
| Serum/urine pregnancy test[l] | X | | | X[g] | | | | | | | X |
| Concomitant medications[m] | Collected through the study | | | | | | | | | | |
| Adverse events[n] | Collected through the study | | | | | | | | | | |
| Blood Phe/Tyr levels (DBS)[o] | X | Days 1, 4, and 7 | X | X | X | X | X | X | X | X | X |
| Palatability assessments (<18 years only)[p] | | | | X | | | | | | | |
| Consistent diet/diet monitoring/3-day diet record[q] | X[r] | X | | X | | | | | | | |
| Dose study drug | | | | Daily for 14 days | | | | | | | |

| Evaluation | Screening Period (Up to a Total of 45 Days) | | Part 1 - Open-Label Responsiveness Test | | | | | | | | ETV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screening Visit | $BH_4$ Washout Period | Sepiapterin Open-Label Treatment 60 mg/kg | | | | | Sepiapterin Washout | | | |
| | Up to 45 Days | 7 Days[a] | 14 Days | | | | | Min 14 Days Max 21 Days | | | |
| **Study Day** | | | **-1** | **1** | **7** | **10** | **14** | **19** | **24** | **28[b]** | |
| Dispense study drug | | | | X[s] | | | | | | | |
| Collect study drug, assess compliance | | | | | | | X[s] | | | | X |

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

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

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

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

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

[e] Part 1 Day 14 virtual visit: A +1-day window is permitted. Part 1 Day 28 (up to Day 35): telephone call to confirm eligibility for continued participation and to schedule either Part 2 Day 1 Visit or ETV.

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

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

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

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

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

[k] eGFR should be calculated for all participants.

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

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

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

[o] Blood Phe/Tyr levels via dried blood sample will be measured at each timepoint indicated. If $BH_4$ Washout Period Day 7 is the same day as Day -1 of Part 1, only 1 dried blood sample each for Phe/Tyr should be collected. If Part 1 Day 28 (sepiapterin washout) is the same day as Part 2 Treatment Period 1 Day -1, only 1 dried blood sample each for Phe/Tyr should be collected. Samples will be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day at each collection timepoint. Blood Phe/Tyr samples can be collected while the participant is either in the clinic or at home. Samples obtained at home will be shipped to the study site. Analysis of the screening blood Phe level can be performed at the site's local laboratory for determination of eligibility; however, a separate sample for blood Phe/Tyr should still be sent to the central laboratory for the screening timepoint. Vital signs should be collected prior to blood Phe/Tyr collection at the Screening Visit.

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

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

[r] Participants with a >20% variance in dietary Phe consumption during the 24- to 30-day Dietary Control Observation Period will be considered screen failures.

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

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

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

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

| Evaluation | Part 2 - Randomized, Active-Controlled Open-Label, Crossover Treatment Period | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment Period 1 Sepiapterin 60 mg/kg OR 20 mg/kg Sapropterin 28 Days (4 Weeks) | | | | | | | | Washout Period 1 (14 Days)[a] | Treatment Period 2 Sepiapterin 60 mg/kg OR 20 mg/kg Sapropterin 28 Days (4 Weeks) | | | | | | | | Washout Period 2 (14 Days)[a] | EOS[b] | ETV[c] |
| **Study Day** | **-1** | **1** | **7** | **10** | **14** | **19** | **24** | **28** | **1-14** | **-1** | **1** | **7** | **10** | **14** | **19** | **24** | **28** | **1-14** | | |
| Randomization | X[d] | | | | | | | | | | | | | | | | | | | |
| In-clinic visit | | X[e] | | | | | | X[e] | | | X[e] | | | | | | X[e] | | X | X |
| Virtual visit | | | | | X[e] | | | | | | | | | X[e] | | | | | | |
| Vital signs,[f]weight | | X[g] | | | | | | X | | | X[g] | | | | | | X | | X | X |
| Height[h] | | | | | | | | X[c] | | | | | | | | | X[c] | | X[c] | |
| ECGs[i] | | | | | | | | X | | | | | | | | | X | | X | X |
| Physical examination[j] | | X[g] | | | | | | X | | | X[g] | | | | | | X | | X | X |
| Clinical laboratory tests[k] | | X[g] | | | | | | X[g, l] | | | X[g] | | | | | | X[l] | | X[l] | X |
| Urine pregnancy test[m] | | X[g] | | | | | | X | | | X[g] | | | | | | X | | X | X |
| Concomitant medications[n] | Collected throughout the study | | | | | | | | | | | | | | | | | | | |
| Adverse events[o] | Collected throughout the study | | | | | | | | | | | | | | | | | | | |
| Blood Phe/Tyr levels (DBS)[p] | X | X | X | X | X | X | X | X | Days 5, 10, and 14 | X | X | X | X | X | X | X | X | Days 5, 10, and 14 | X | X |
| Consistent diet/diet monitoring/3-day diet record[q] | X | | | | | | | | | | | | | | | | | | | X |
| QOL assessments[r] | | X | | | | | | X | | | | | | | | | X | | | |
| Dose study drug | | Daily for 28 days (4 weeks) | | | | | | | | | Daily for 28 days 4 weeks) | | | | | | | | | |
| Dispense study drug[s] | | X | | | | | | | | | X | | | | | | | | | |
| Collect study drug, assess compliance[s] | | X | | | | | | X | | | X | | | | | | X | | | X |

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

[a] The washout periods in Part 2 consist of 14 days (+3-day window). During the washout periods, study drug will not be administered, and compliance will not be assessed. It is acceptable for Day 14 of the Washout Period 1 and Day -1 of TP2 to be performed on the same day.

[b] An EOS Visit will be performed on/after Day 14 (+3 days) of Washout Period 2.

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

[d] Randomization can occur anytime during the Part 1 sepiapterin washout after confirmation of meeting sepiapterin responsiveness of blood Phe reduction ≥20% and scheduling of Part 2 Treatment Period 1 Day 1 in-clinic visit.

[e] In-clinic visits: In-clinic visits will occur for all participants on Part 2 Day 1 and Part 2 Day 28 for each treatment period. There is a ±2-day window permitted for the Part 2 Day 28 Visit for each treatment period. Participant should continue treatment until the Day 28 (±2 days) Phe sample has been collected. Virtual visits: A ±1-day window is permitted for all virtual visits.

[f] Includes blood pressure, pulse, respiratory rate, and temperature. Obtain vital signs prior to collection of any laboratory samples (including Phe). If feasible, participants will rest for 5 minutes in a supine position before vital signs are assessed. Vital signs will be collected both predose and 2 hours postdose on Part 2 Day 1 during each treatment period; for all other timepoints, they will be taken at any time during the visit but prior to collection of any laboratory samples (including Phe).

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

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

[i] If feasible, participants will rest for 5 minutes in a supine position before ECG is performed. Twelve-lead ECGs will be performed on Part 2 Day 28 for each treatment period and EOS and at the ETV (if appropriate). Electrocardiograms will be performed in triplicate, with each read taken at least 1 minute apart.

[j] Conduct a complete physical examination of general appearance, dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, and neurological 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, and uric acid), hematology panel (hematocrit, hemoglobin, platelet count, RBC count, WBC count, and WBC differential), and urinalysis (bilirubin, glucose, ketones, occult blood, pH, protein, specific gravity, urobilinogen, and microscopic examination of WBCs, RBCs, and epithelial cells). Participants should be fasted prior to collection of samples (minimum of 4 hours for participants <6 years and minimum of 8 hours for participants ≥6 years).

[l] eGFR should be calculated (all participants).

[m] Pregnancy tests are required for all women of childbearing potential; urine pregnancy testing to occur on Days 1 and 28 of each treatment period, and urine testing to occur during all other in-clinic visits. Any positive urine pregnancy test should be confirmed by a serum pregnancy test performed at the site's local laboratory.

[n] Record all treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) starting from 30 days prior to Screening until completion of EOS or ETV (if applicable).

[o] Adverse events will be collected from the time of informed consent and/or assent (as applicable) until completion of EOS or ETV (for participants who discontinue prematurely or are considered nonresponsive to sepiapterin after Part 1; participants will continue to be followed for 30 days after last dose for SAEs). If a participant elects not to continue into Study PTC923-MD-004-PKU, they should have a follow-up phone call 30 (±3) days after their EOS Visit for collection of AEs.

[p] Blood Phe/Tyr levels via dried blood sample are measured at each timepoint indicated. Day -1 and Day 1 (predose) samples should be taken predose. All other samples can be collected predose or postdose. If Part 1 Day 28 (sepiapterin washout) is the same day as Part 2 Treatment Period 1 Day -1, only 1 dried blood sample for Phe/Tyr should be collected. Part 1 Day 28 samples would not be collected in this circumstance. If Part 2 Washout Period 1 Day 14 is the same day as Day -1 of Part 2 Treatment Period 2, only 1 dried blood sample for Phe/Tyr should be collected. The Part 2 Washout Period 1 Day 14 samples would not be collected in this circumstance. Samples will be collected after fasting or no earlier than 3 hours postprandial and at approximately the same time of day at each collection timepoint. Samples obtained at home will be shipped to the study site or returned to the study site at the next clinic visit. Samples are 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 of ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.

[q] Participants are to maintain a consistent diet (with respect to Phe intake) during the study. At each in-clinic visit, a dietitian will monitor each participant's diet to calculate Phe consumption and to have regular contact with participants. The 3-day diet records will be collected biweekly during the Part 2 Treatment and Washout Periods.

[r] 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]) will be conducted using the tool in one of the following validated languages: English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the PKU-QOL will not be required for participants whose primary language is not one of the available validated languages. EQ-5D (EQ-5D-Y Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and EQ-5D-5L [≥16 years]) will also be conducted.

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

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

## TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| ACMG | American College of Medical Genetics |
| AE | Adverse event |
| ALT | Alanine aminotransferase |
| AP | Alkaline phosphatase |
| AST | Aspartate Aminotransferase |
| $BH_4$ | Tetrahydrobiopterin |
| BUN | Blood urea nitrogen |
| COVID-19 | Coronavirus disease 2019 |
| CRO | Contract Research Organization |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DBS | Dried blood sample |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| EQ-5D | European Quality of Life - 5 Dimensions |
| EQ-5D-5L | 5-Level European Quality of Life - 5 Dimensions |
| EQ-5D-Y | European Quality of Life - 5 Dimensions for Youth |
| ETV | Early Termination Visit |
| EU | European Union |
| FAS | Full Analysis Set |
| FAS | Full Analysis Set |
| 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 Committee |
| IRB | Institutional Review Board |
| IRT | Interactive Response Technology |
| LDH | Lactate dehydrogenase |
| LS | Least squares |
| MAR | Missing at random |
| MI | Multiple imputation |
| MMRM | Mixed model repeated measures |
| NDA | New Drug Application |
| PAH | Phenylalanine hydroxylase |
| PAS | Primary Analysis Set |
| Phe | Phenylalanine |
| 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 |
| SAP | Statistical analysis plan |
| SmPC | Summary of Product Characteristics |
| SoC | Standard of care |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TEAE | Treatment-emergent adverse event |
| TP | Treatment Period |
| Tyr | Tyrosine |
| USM | Urgent safety measure |
| VAMS | Volumetric absorptive microsampling |
| Vol | Volume |
| WBC | White blood cell |

# 2. INTRODUCTION

## 2.1. 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 and Kaufman 2001). Gene mutations of PAH result in decreased catalytic activity leading to hyperphenylalaninemia (HPA) (Al Hafid and Christodoulou 2015). There are many different mutations in the *PAH* gene (>1600), 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 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, are associated with cognitive dysfunction and memory impairment, and can lead to psychiatric and behavioral problems (Kaufman 1989). If left untreated, severe and irreversible intellectual disability can occur (Scriver and Kaufman 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).

## 2.2. 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 foods such as meat, fish, milk, cheese, bread, nuts, and many other common food items. Even the intake of vegetables and fruit are limited.

The success of dietary control, however, comes at a 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 the 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.

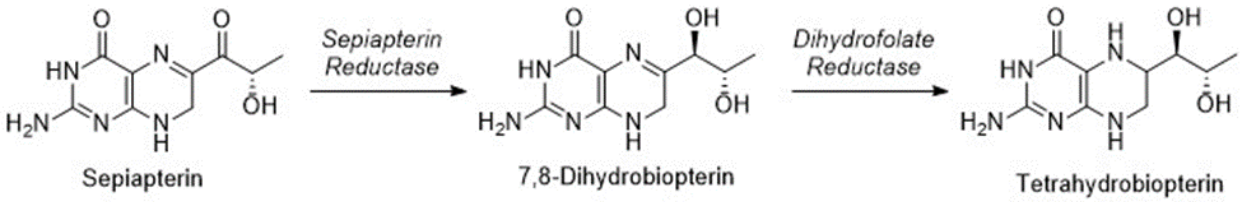
Synthetic tetrahydrobiopterin ($BH_4$) (eg, sapropterin) is commercially available as an approved drug for the treatment of HPA in PKU (FDA New Drug Application [NDA] 22181, EMEA/H/C/000943). Sapropterin is administered orally, once daily at a dose of 5 to 20 mg/kg (Kuvan SmPC 2020, KUVAN USPI 2021). 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, 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 approved in 2018 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 >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, Phe 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).

## 2.3. Sepiapterin

Sepiapterin (formerly known as PTC923 and CNSA-001) is a new molecular entity, an exogenously synthesized, structurally equivalent version of the biologically produced compound sepiapterin. Endogenous sepiapterin serves as a substrate for de novo synthesis of $BH_4$ via the pterin salvage pathway (Mayer and Werner 1995), making sepiapterin a naturally occurring precursor for $BH_4$ (Figure 2). Tetrahydrobiopterin is an essential cofactor for enzymes including PAH (Kaufman 1989), tyrosine 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, sepiapterin is rapidly converted to $BH_4$ intracellularly (Smith 2019), 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 that 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 2: Endogenous Metabolism of $BH_4$ From Sepiapterin**




**Abbreviations**: $BH_4$, tetrahydrobiopterin

## 2.4. Study Rationale and Dose Justification

Currently, there is no cure for PKU. Control with dietary Phe restriction and/or $BH_4$ supplementation is suboptimal. Tetrahydrobiopterin supplementation with sepiapterin 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). Sepiapterin 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 of escalating doses of sepiapterin administered orally to healthy volunteers. This study showed that sepiapterin 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 sapropterin across equivalent doses (Smith 2019), which would be expected to result in improved efficacy. Following this, Study PKU-002, a Phase 2 study conducted in participants with PKU, provided a direct head-to-head comparison of sepiapterin and sapropterin with a dose comparison of the effect of 20 mg/kg/day sapropterin versus 20 mg/kg/day sepiapterin versus 60 mg/kg/day sepiapterin. In Study PKU-002, sepiapterin 60 mg/kg was significantly more effective than sapropterin 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 sepiapterin 60 mg/kg on blood Phe was approximately 1.4× larger than that of sepiapterin 20 mg/kg, driving dose decisions for future studies. There were no notable differences in the safety profile of sepiapterin and sapropterin. Study PKU-002 provided proof of the change in efficacy of sepiapterin with statistically superior reductions in blood Phe levels versus sapropterin and helped to establish dose selection for this study.

Study PTC923-PKU-301 is a Phase 3 active comparator study to assess the efficacy of the to-be-marketed dose of sepiapterin (60 mg/kg/day) in reducing blood Phe levels compared with the maximum recommended daily dose of sapropterin (20 mg/kg/day) in participants with PKU. Based on the results from Study PKU-002, it is anticipated that a greater reduction in Phe will be observed in participants with PKU after 4 weeks of treatment with sepiapterin versus after 4 weeks of treatment with sapropterin. This Phase 3 study is designed to support registration of sepiapterin in participants with PKU.

## 2.5. Study Duration

The study duration for each participant will be up to 173 days from Screening to the final study visit. Following a screening period of up to 45 days, all enrolled participants will receive sepiapterin for 2 weeks followed by a minimum 14-day (maximum 21-day) sepiapterin washout period in Part 1. Participants with ≥20% reduction in blood Phe levels to sepiapterin will continue into Part 2 where they will receive sequential treatment with 4 weeks of sepiapterin and 4 weeks of sapropterin (order determined by randomization) within each treatment period. Following completion of each 4-week treatment period, participants will complete a washout (14 days [+3-day window]). After completion of the second 14-day washout period, participants will be offered the option to enroll directly into an open-label long-term study, PTC923-MD-004-PKU.

## 2.6. Benefit/Risk Assessment

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 participants and veracity of data collected.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of sepiapterin can be found in the current version of the Investigator's Brochure and associated Reference Safety Information (RSI).

The benefits and risks of KUVAN for patients with PKU are already established and outlined in the KUVAN Summary of Product Characteristics (SmPC) (Kuvan SmPC 2020).

Clinical knowledge exists for sepiapterin as it has been evaluated in more than 388 participants in the completed and ongoing clinical studies including >180 participants with PKU. Across all studies, sepiapterin has been well tolerated and exhibited a favorable safety profile with manageable side effects.

### 2.6.1. Eligible Study Population

As per Inclusion Criterion 3, eligible participants for this study have PKU with blood Phe level ≥360 µmol/L anytime during Screening and blood Phe level ≥360 µmol/L on current therapy. As such, this study has the potential to benefit adult and pediatric PKU patients, since participation offers an opportunity for study treatment to reduce blood Phe levels to within accepted guidelines.

Sepiapterin 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 adult participants with PKU (Bratkovic 2022). Furthermore, in participants with classical PKU, the reduction in mean blood Phe with sepiapterin was also larger than that observed with sapropterin. In the recently completed Phase 3 Study PTC923-MD-003-PKU, a highly statistically significant and clinically relevant reduction in blood Phe levels was demonstrated for adult and pediatric sepiapterin-treated subjects across all disease severities (ie, mild, moderate, and severe [classical] PKU).

Consistent with global regulation and guidelines with inclusion of minors in clinical studies (eg, Clinical Trial Regulation article 32, litra e and f), inclusion of pediatric patients in this clinical study is appropriate given that diagnosis of PKU, an inborn error of metabolism, occurs at birth via newborn screening and the necessity of starting dietary protein/Phe restriction and or pharmacotherapy shortly after birth to prevent severe and irreversible debilitating neurological deficits from chronically elevated blood Phe is mandatory. As part of the sepiapterin clinical development program, the Phase 3 randomized double-blind placebo-controlled Study PTC923-MD-003-PKU enrolled approximately 60% of study participants who were <18 years (the youngest being 1 year) of age and utilized an initial 14-day period of sepiapterin responsiveness testing, similar to Part 1 of this protocol. This limited period of initial exposure to sepiapterin mitigates against exposure of participants to a potentially non-efficacious treatment. For participants who are identified as responsive to sepiapterin, the 2 treatment periods in Part 2 are of a sufficient duration (4 weeks each) to effectively assess efficacy of sepiapterin compared to sapropterin.

As such, participation in this clinical study is likely to have a direct and significant benefit for children, adolescent, and adult PKU patients that outweighs the risks and burdens involved.

### 2.6.2. Study Design and Procedures

#### *2.6.2.1. Monitoring of Blood Phe*

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 and repeatable, and the results are comparable to those of a conventional venous blood sampling human plasma HPLC/MS/MS method (Gao 2023). Samples collected in the Phase 2 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 to reduce the number of clinic visits and increase convenience to participants.

#### *2.6.2.2. Exposure of Nonresponsive Participants*

The primary objective of this study is to test whether sepiapterin is superior to sapropterin in terms of blood Phe reduction. For this reason, Part 1 of the study allows for an all-comer population, which could include treatment-naive, sapropterin-responsive, and sapropterin-nonresponsive subjects to be tested for responsiveness to sepiapterin only. To reduce exposure of nonresponsive participants to sepiapterin, in Part 1, all eligible participants will be tested for responsiveness to sepiapterin. Participants who experience <20% reduction in blood Phe levels will be classified as nonresponsive, and participation in the study will be terminated after minimal exposure to sepiapterin. A reasonable threshold to define treatment responsiveness in patients with PKU was a ≥30% reduction in blood Phe concentration to allow inclusion in the Primary Analysis Set (PAS). This was based on historical use in other clinical studies of sepiapterin and sapropterin, including the completed pivotal Phase 3 Study PTC923-MD-003-PKU (Burton 2007). In addition, as per the KUVAN SmPC, "A satisfactory response is defined as a ≥30 percent reduction in blood phenylalanine levels or attainment of the therapeutic blood phenylalanine goals defined for an individual patient by the treating physician. Patients who fail to achieve this level of response within the described one-month test period should be considered nonresponsive, these patients should not be treated with KUVAN and administration of KUVAN should be discontinued."

It is important to note that not all patients who have PKU have been previously assessed for responsiveness to sapropterin and, in the event responsiveness testing has been performed, the duration is highly variable, ranging from 24 hours to 30 days. Accordingly, sapropterin responsiveness is not an inclusion/exclusion criterion for study participation. The study design accounts for the possibility that sapropterin-responsive patients will be enrolled in the study by including an appropriate washout period during screening for these patients in preparation for the randomized withdrawal design of this crossover study. Only after participants who receive the test treatment sepiapterin demonstrate a response for the specified 2-week duration (and after sufficient washout from sapropterin, if required) will the participants be randomized to receive either test treatment or control drug in Part 2.

#### *2.6.2.3. Pausing and Washing Out of Regular PKU Therapy*

During this study, participants will be required to pause and/or wash out their regular pharmacological therapy for PKU. It is not anticipated that pausing, washout, and restarting of study treatment is likely to pose a potential risk for participants in relation to change in blood Phe levels. The potential for "rebound" effects, defined as an increase in blood Phe levels above pretreatment levels, will be monitored throughout the study by regular blood Phe measurements.

For those participants who have a pharmacologic therapy in the screening period, pausing and/or washing out of regular treatment may temporarily result in worsening of a participant's HPA in a similar manner to what might be experienced when ill with a cold or the flu. Restarting regular treatment may result in symptoms such as headaches, anxiety, or lethargy as participants' HPA rapidly reduces to target therapeutic concentrations. Blood Phe will be monitored throughout the washout and treatment periods in this study to ensure against prolonged increases in HPA. Of note, for those participants entering the study on dietary management only, their blood Phe during washout periods is expected to return to baseline levels in the screening period. Both sepiapterin and sapropterin have demonstrated the ability to lower blood Phe in patients with PKU in clinical studies (Burton 2007, Bratkovic 2022). Therefore, the use of sepiapterin as a treatment and sapropterin as a control is scientifically justified and mitigates the potential risks associated with pausing, washout, and restart of regular treatment.

#### *2.6.2.4. Conclusion*

Taking into account the measures taken to minimize risk to participants participating in this study, the potential risks identified in association with sepiapterin are justified by the anticipated benefits that may be afforded to participants with PKU. Additionally, all participants will be eligible upon their completion of Study PTC923-PKU-301 to enter the extension Study PTC923-MD-004-PKU that allows for long-term treatment with sepiapterin and also the opportunity to assess treatment effects on dietary Phe tolerance.

# 3. OBJECTIVES AND ENDPOINTS

The study objectives and associated endpoints are detailed in Table 3.

**Table 3: Objectives and Endpoints**

| Objectives | Endpoints |
|---|---|
| Primary | |
| • To compare the efficacy of sepiapterin to sapropterin in reducing blood Phe levels in participants with PKU | • Mean change in blood Phe levels from baseline to Weeks 3 and 4 of each treatment period (the average of the last 2 weeks of each treatment period) in Part 2 |
| Secondary | |
| • To evaluate the efficacy of sepiapterin in reducing blood Phe levels<br>• To assess the safety and tolerability of sepiapterin | • Proportion of participants with baseline blood Phe levels ≥600 µmol/L who achieve Phe levels <600 µmol/L after each treatment period in Part 2<br>• Proportion of participants reaching blood Phe <360 µmol/L after each treatment period in Part 2<br>• AEs, physical examinations, vital sign assessments, 12-lead ECGs, and routine clinical laboratory assessments |
| Exploratory | |
| • To evaluate changes in blood Tyr over time, including the Phe:Tyr ratio<br>• To assess the taste, palatability, and acceptability (<18 years) of sepiapterin<br>• To evaluate sepiapterin effect on QOL using the PKU-QOL questionnaire in the subset of participants who are able to complete the PKU-QOL (ie, participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years, Parent PKU-QOL; ages 9 to 11 years, Child PKU-QOL; ages 12 to 17 years Adolescent PKU-QOL; and ages ≥18 years Adult PKU-QOL)<br>• To evaluate sepiapterin effect of QOL using the EQ-5D (EQ-5D-Y Proxy Version 1 [3 to 7 years]; EQ-5D-Y [8 to 15 years]; and EQ-5D-5L ([≥16 years]) | • Changes in blood Tyr over time, including the Phe:Tyr ratio<br>• Taste, palatability, and acceptability scores (<18 years)<br>• Changes from baseline in QOL using PKU-QOL questionnaire in the subset of participants that are able to complete the PKU-QOL (ie, participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French) (ages 6 to 8 years, Parent PKU-QOL; ages 9 to 11 years, Child PKU-QOL; ages 12 to 17 years, Adolescent PKU-QOL; and ages ≥18 years Adult PKU-QOL)<br>• Changes from baseline in QOL using the 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]) |

**Abbreviations**: AE, adverse event; ECG, electrocardiogram; 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; Phe, phenylalanine; PKU, phenylketonuria; PKU-QOL, Phenylketonuria-Quality of Life; QOL, quality of life; Tyr, tyrosine

# 4. STUDY DESIGN

## 4.1. Overall Design

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

During this study, participants should continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or dietary Phe consumption) as measured by 3-day diet record collection (weekly during Screening and Part 1 Treatment Period; biweekly during Part 1 Washout Period and Part 2 Treatment and Washout Periods). The diet should remain unchanged throughout the study, regardless of treatment or washout periods. The study schema is provided in Figure 1.

### 4.1.1. Screening Period (up to 45 Days)

#### *4.1.1.1. General Procedures*

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), participants will undergo in-clinic screening procedures to determine study eligibility. Participant 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 participant is known to be $BH_4$ responsive). Vital signs, weight, and height measurements will be performed. Phenylalanine hydroxylase (*PAH*) gene genotyping should be performed unless already documented in the participant's medical history. Blood Phe/tyrosine (Tyr) levels will be measured. A full physical examination will be performed, and urine 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). Participants will be instructed to continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) throughout the entire study. Participants will maintain a 3-day diet record during each week of Screening, and a dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification. Participants who enter the study and are receiving $BH_4$ supplementation (eg, sapropterin, KUVAN) at the Screening Visit must complete a 7-day washout period prior to dosing.

#### *4.1.1.2. Dietary Control Observation Period (24 to 30 Days)*

Participants will continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) and will maintain a weekly 3-day diet record for 4 consecutive weeks during the Dietary Control Observation Period. The diet should remain unchanged throughout the study, regardless of treatment or washout periods. Participants with a >20% variance in dietary Phe consumption during the 24- to 30-day Dietary Control Observation Period will be considered screen failures.

#### *4.1.1.3. $BH_4$ Supplementation Washout (7 Days)*

Following the initial Screening Visit, eligible participants who are taking $BH_4$ supplementation must discontinue the medication as concomitant treatment/supplementation with $BH_4$ will not be permitted during the study. Participants will continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) and will maintain a 3-day diet record during the $BH_4$ Supplementation Washout. A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants 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).

### 4.1.2. Part 1, Open-Label Responsiveness Test and Sepiapterin Washout (28 to 35 Days)

#### *4.1.2.1. Part 1, Open-Label Treatment (14 Days)*

Following the initial screening and $BH_4$ Supplementation Washout (as necessary), all eligible participants will be enrolled into Part 1 of the study to test for responsiveness to sepiapterin. Participants will receive sepiapterin 60 mg/kg for 14 days.

On Part 1 Day 1, participants will take their dose of sepiapterin while in the clinic; for all other dosing days, participants will take their dose as an outpatient and prior to coming to the clinic when visits are scheduled. All visits will be in-clinic visits or virtual visits (unless specified as a mandatory in-clinic visit). Study visits will occur on Part 1 Day 1 and Part 1 Day 14. On Day 1, palatability and acceptability assessments will be performed for all participants <18 years of age: Palatability will be indirectly assessed by the parent(s)/caregiver(s) of participants <5 years of age; taste and palatability will be assessed separately by participants ≥5 to <18 years; acceptability will be assessed by parent(s)/caregiver(s) for all children <12 years of age.

Blood Phe levels will be measured on Days -1 (only 1 sample is required if this is on the same day as $BH_4$ Washout Period Day 7), 1 (predose), 7, 10, and 14 of Part 1. Baseline blood Phe level will be calculated as the mean of the blood Phe levels from dried blood samples taken on Day -1 and Day 1 (predose). Blood Phe samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.

Participants will continue their usual diet without modification and will maintain 3-day diet records (ie, 1 corresponding to each week of the sepiapterin open-label treatment period). A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.

#### *4.1.2.2. Part 1, Sepiapterin Washout (14 Days Minimum/21 Days Maximum)*

Following completion of 14 days of sepiapterin open-label treatment, participants will begin a minimum 14-day (maximum 21-day) washout. No in-clinic study visits are required during the sepiapterin washout. In Part 1, blood Phe levels will be measured on Days 19, 24, and 28 during the sepiapterin washout. If Part 1 Day 28 is the same day as Part 2 Treatment Period 1 Day -1, only 1 dried blood sample each for Phe/Tyr should be collected (the Part 1 Day 28 samples would not be collected under these circumstances). 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. Participants will continue their usual diet without modification and will maintain a 3-day diet record (ie, 1 corresponding to each 2-week period of the sepiapterin washout). A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.

During the sepiapterin washout, the mean change in blood Phe levels over the 14 days of sepiapterin treatment (mean of Part 1 Days 7, 10, and 14) will be measured and compared against their pretreatment blood Phe level (mean of Part 1 Day -1 and Part 1 Day 1 [predose]). Determination of a participant's sepiapterin responsiveness may be conducted with ≤2 pretreatment blood Phe levels (but requiring a minimum of 1 pretreatment level) and ≤3 sepiapterin treatment blood Phe levels (but requiring a minimum of 1 sepiapterin treatment level), depending on the available data on or between Part 1 Study Day 28 and Day 35.

Participants who experience <20% 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 and Day 35).

Part 1 for all participants ends on Day 35. In no circumstances will a participant be permitted to continue in Part 1 beyond Day 35. If a participant's blood Phe concentrations are not available (ie, a minimum of 1 pretreatment level and 1 sepiapterin treatment level) for determination of responsiveness to sepiapterin by Day 35 of Part 1, then that participant 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 long-term study, PTC923-MD-004-PKU.

### 4.1.3. Part 2, Randomized, Active-Controlled, Open-Label, Crossover Treatment Period (84 Days)

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

For all days where in-clinic visits are scheduled, participants will take their dose of study drug (sepiapterin or sapropterin) while in clinic. A 14-day washout period (+3-day window) occurs after each treatment period (Washout 1 and Washout 2). All visits will be in-clinic visits or virtual visits (unless specified as a mandatory in-clinic visit). For each treatment period, study visits will occur on TP1 Day 1, TP1 Day 28, TP2 Day 1, and TP2 Day 28 to conduct study evaluations, collect 3-day diet records, and capture any AEs and concomitant medications.

Participants will continue their usual diet without modification and will maintain 3-day diet records (ie, 1 corresponding to each 2-week period of Part 2). A dietitian will monitor each participant's diet to calculate total protein and corresponding Phe consumption, to have regular contact with participants, and to reinforce the need for participants to maintain their usual diet without modification.

During each treatment period, blood Phe levels will be measured in Part 2 on Days -1, 1 (predose), 7, 10, 14, 19, 24, and 28. Baseline is the mean of Day -1 and Day 1 predose blood Phe levels, and the mean blood Phe levels of every 2-week intervals will be calculated (Days 7, 10, and 14 for Weeks 1 and 2 and Days 19, 24, and 28 for Weeks 3 and 4). 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. If Part 2 Washout Period 1 Day 14 is the same day as Part 2 Treatment Period 2 Day -1, only 1 dried blood sample each for Phe/Tyr should be collected (the Part 2 Washout Period 1 Day 14 samples would not be collected under these circumstances).

On Part 2 Day 1 of TP1 and Day 28 of TP1 and TP2, 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]; and Adult PKU-QOL [≥18 years]) will be conducted using the tool in one of the following validated languages: English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the PKU-QOL will not be required for participants whose primary language is not one of the available validated languages. 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.

### 4.1.4. End of Study Visit

For all participants who complete Part 2, End of Study (EOS) is defined as their final study visit on completion of the 14-day washout (+3-day window) after Treatment Period 2. All participants who complete Part 2 will be given the opportunity to continue treatment in the sepiapterin open-label long-term study, PTC923-MD-004-PKU. If a participant elects not to continue into Study PTC923-MD-004-PKU, they should have a follow-up phone call 30 (±3) days after their EOS Visit for collection of AEs.

Enrollment will continue until 34 participants are randomized into the stratum of ≥30% blood Phe reduction sepiapterin-responsive participants. The EOS is defined as the date of the last visit of the last participant in the study globally.

### 4.1.5. Early Termination Visit

An ETV will be performed on or between Day 28 and Day 35 for all participants who are determined to be nonresponsive to sepiapterin in Part 1 or within 3 days for any participant who discontinues the study prematurely during Part 1 or Part 2 for any other reason. During the ETV, completed 3-day diet records, AEs, concomitant medications, weight, vital signs, electrocardiograms (ECGs), and blood Phe/Tyr levels will be collected. A physical examination, clinical laboratory tests, and a pregnancy test will be performed. For nonresponsive participants and participants who discontinue prematurely, following the ETV, participants may revert to their prestudy treatment for PKU (at the investigator's and the participant's treating physician's discretion), and a follow-up phone call will occur 30 (±3) days after the last dose of study drug to assess for serious adverse events (SAEs).

## 4.2. Number of Participants and Study Centers

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

Approximately 18 study centers across Australia, Canada, Czech Republic, Denmark, France, Georgia, Germany, Italy, Netherlands, Poland, Slovenia, Spain, and United Kingdom will participate in this study.

## 4.3. Treatment Assignment

### 4.3.1. Part 1, Open-Label Responsiveness Test

All participants will receive sepiapterin 60 mg/kg for 14 days starting on Day 1.

### 4.3.2. Part 2, Open-Label Crossover Treatment Period

Eligible participants will be randomized to 1 of 2 treatment sequences:

- Sepiapterin 60 mg/kg daily for 4 weeks followed by sapropterin 20 mg/kg daily for 4 weeks (each treatment period followed by a 14-day [+3-day window] washout period)
- Sapropterin 20 mg/kg daily for 4 weeks followed by sepiapterin 60 mg/kg daily for 4 weeks (each treatment period followed by a 14-day [+3-day window] washout period)

## 4.4. Dose Adjustment Criteria

No dose adjustments are permitted during the study (with the exception of the criteria described in Section 4.4.1). All study treatments should be taken as described in Section 4.3.1 and Section 4.3.2.

### 4.4.1. Safety Criteria for Adjustment or Stopping Doses

**Part 2 only**: At the investigator's discretion, it is permitted for participants to temporarily stop treatment once for a period of 7 days (inclusive) if they experience any conditions such as a cold or similar viral or bacterial infection (eg, COVID-19 or otitis media). The purpose of this temporary 7-day hold is to allow for a more accurate reflection of blood Phe levels and typical diet, rather than having falsely elevated blood Phe levels and/or diet changes during times of illness or stress.

**If participants pause treatment on Days 1 to 14**, after the 7-day pause, they should resume treatment on the day they paused originally. For example, if a participant fell ill and stopped treatment on Day 3 of TP1, they should pause for 7 days, and then resume treatment again from Day 3.

**If participants pause treatment on Days 15 to 28**, after the 7-day pause, they should resume treatment on Day 15 by default.

In addition, any blood Phe values collected or diet records during this 7-day period will not be utilized in the study.

Apart from the criteria described above, 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 participant. Safety criteria for permanent discontinuation of study drug are discussed in Section 7.1. If a participant temporarily stops treatment at the occurrence of an AE, the participant may resume at

the previous dose if deemed in the best interest of the participant by the investigator and medical monitor.

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.

## 4.5. 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 participant 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) determines 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 participants to date. In a temporary study hold, no additional participants 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 participants. 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 participants 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.

# 5. STUDY POPULATION

Participants with any PAH mutation are permitted to screen and enroll into the study. However, participants with biochemically diagnosed classical PKU will be capped at 30% of the Safety Analysis Set. Biochemically diagnosed classical PKU includes those with ≥2 historical blood Phe concentrations ≥1200 µmol/L in their medical history. Furthermore, participants with initial newborn screen performed >3 days after birth demonstrating values ≥1200 µmol/L may have these values excluded from the ≥2 historical blood Phe concentration requirement. Genotyping will not be required for study eligibility; however, all participants will undergo genotyping unless documented in their medical history, and these data will be collected for analysis.

## 5.1. Inclusion Criteria

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

Participants are eligible to be included in the study only if all the following criteria apply:

1. Informed consent, and if necessary, assent (with parent/legally designated representative consent)
2. Male or female participants ≥2 years of age
3. Blood Phe level ≥360 µmol/L on current therapy anytime during Screening and blood Phe level ≥360 µmol/L on current therapy when taking the average of the 3 most recent Phe levels from the participant'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 the study drug.

All females will be considered of childbearing potential, ie, fertile, following menarche and until becoming 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 postprocedure.

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

## 5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. The individual, in the opinion of the investigator, is unwilling or unable to adhere to the requirements of the study. Incapacitated adults are not eligible for participation in this study.
2. Gastrointestinal disease (such as irritable bowel syndrome, inflammatory bowel disease, chronic gastritis, 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 any of the ingredients or excipients of 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 participant's ability to participate in the study or increase the risk of participation for that participant

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/1.73 $m^2$

    In participants ≥18 years of age, the Modification of Diet in Renal Disease Equation should be used to determine GFR.

    In participants <18 years, the Bedside Schwartz Equation should be used to determine GFR.
12. Requirement for concomitant treatment with levodopa or 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 guanosine triphosphate 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. Unwillingness to washout from $BH_4$ supplementation (eg, sapropterin, KUVAN)
16. Use of pegvaliase-pqpz (PALYNZIQ) concurrently or within the 60 days prior to Screening
17. Greater than 20% variance in dietary Phe consumption as measured by mandatory weekly 3-day diet record collection for 4 consecutive weeks (Dietary Control Observation Period during Screening)

### 5.3. Lifestyle Considerations

During this study, participants should continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption) as measured by 3-day diet record collection (weekly during Screening and Part 1 Treatment Period; biweekly during Part 1 Washout Period and Part 2 Treatment and Washout Periods). The diet should remain unchanged throughout the study, regardless of treatment or washout periods.

### 5.4. Screen Failures

Any participant 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.

# 6. STUDY INTERVENTION(S) AND CONCOMITANT THERAPY

In Part 2 of the study, participants will be randomized 1:1 to receive either:

- Sepiapterin 60 mg/kg/day for 4 weeks followed by sapropterin 20 mg/kg/day for 4 weeks
- Sapropterin 20 mg/kg/day for 4 weeks followed by sepiapterin 60 mg/kg/day for 4 weeks

Dosing of sepiapterin and sapropterin is based on the participant's weight. The weight obtained on Day 1 of each treatment period will be used to calculate the exact amount (in mg) of each treatment required for each participant's daily dose.

Details on preparation of sepiapterin and sapropterin and dosing guidelines will be provided in the Pharmacy Manual for the site and an instruction guide for participants.

## 6.1. Study Interventions Administered

### 6.1.1. Sepiapterin (Test Drug)

Sepiapterin is a yellow to orange powder that is available in two strengths, 250 mg or 1000 mg, and is intended to be suspended in water or apple juice or mixed in soft foods such as apple sauce, strawberry jam, or jello.

### 6.1.2. Sapropterin (Active Control)

The active control is sapropterin 100 mg tablets. For posology, please refer to the KUVAN SmPC (Kuvan SmPC 2020).

## 6.2. Selection and Timing of Dose for Each Participant

The appropriate assigned study treatment (sepiapterin or sapropterin, dependent on randomization) 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, participants will take their morning dose as an outpatient with a meal.

## 6.3. Preparation, Handling, Storage, and Accountability

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

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. The study drug must be disposed of (destroyed) by incineration. Records documenting the date of study drug destruction or shipping and amount destroyed or shipped should be kept.

## 6.4. Randomization and Blinding

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

Following the minimum 14-day (maximum 21-day) sepiapterin washout period, all eligible participants will proceed to Part 2 and be randomized 1:1 to 1 of 2 treatment sequences (sepiapterin-sapropterin OR sapropterin-sepiapterin).

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

## 6.5. 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.

Given the clear physical differences between sepiapterin and sapropterin, full blinding is not possible. However, as much as possible, PTC will remain blinded to treatment sequence until after database lock.

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

## 6.6. Total Blood Volume

The total volume of blood that will be collected from each participant for Screening, Part 1, and Part 2 of the study is presented in Table 4, Table 5, and Table 6, 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 timepoint and 3% of total blood volume during a period of 4 weeks (European Parliament 2008). Total blood volume is estimated to be 80 to 90 mL/kg body weight. Participants of all ages are permitted for inclusion into this study. Based on growth charts for participants aged 2 years, 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 participants aged 2 years would be 8.2 mL at a single time and 24.5 mL over 4 weeks.

To adhere to the guidance, two groups based on age are represented with corresponding blood volumes in Table 4, Table 5, and Table 6.

Screening, consisting of the Screening Visit and $BH_4$ Washout Period, may last up to 45 days (6 weeks). If a participant weighs less than 4.5 kg 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 4: Blood Volume - Screening (Screening Visit and Supplementation Washout Period)**

| | Participants ≥12 Years | | Participants ≥2 Years to <12 Years | |
|---|---|---|---|---|
| | **Vol × Frequency** | **Total Vol** | **Vol × Frequency** | **Total Vol** |
| Blood chemistry | 5.0 mL×1 | 5.0 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 (including serum pregnancy test in female participants [if applicable]) | 3.5 mL×1 | 3.5 mL | | |
| Blood Phe and Tyr levels | 0.16 mL×1 | 0.16 mL | 0.16 mL×1 | 0.16 mL |
| *PAH* genotyping[a] | 2.0 mL×1 | 2.0 mL | 2.0 mL×1 | 2.0 mL |
| Blood Phe and Tyr levels during Supplementation Washout | 0.16 mL×3 | 0.48 mL | 0.16 mL×3 | 0.48 mL |
| **Total Blood Volume** | | **14.14 mL** | | **5.04 mL** |

**Abbreviations**: PAH, phenylalanine hydroxylase; Phe, phenylalanine; PKU, phenylketonuria; Tyr, tyrosine; Vol, volume
[a] Required only if genotyping is not part of PKU medical history (Section 8.3.8).

Part 1, consisting of the open-label sepiapterin responsiveness test and sepiapterin washout period, may last up to 35 days.

**Table 5: Blood Volume - Part 1 (Open-Label Responsiveness Test Period)**

| | Participants ≥12 Years | | Participants ≥2 Years to <12 Years | |
|---|---|---|---|---|
| | **Vol × Frequency** | **Total Vol** | **Vol × Frequency** | **Total Vol** |
| Blood chemistry | 5.0 mL×1 | 5.0 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 (including serum pregnancy test in female participants [if applicable]) | 3.5 mL×1 | 3.5 mL | | |
| Blood Phe and Tyr levels | 0.16 mL×8 | 1.28 mL | 0.16 mL×8 | 1.28 mL |
| **Total Blood Volume** | | **12.78 mL** | | **3.68 mL** |

**Abbreviations**: Phe, phenylalanine; Tyr, tyrosine; Vol, volume

Part 2, consisting of the randomized, active-controlled, open-label, crossover treatment period, may last up to 84 days (12 weeks).

**Table 6: Blood Volume - Part 2 (Open-Label Treatment Period) and End of Study**

| | Participants ≥12 Years | | Participants ≥2 Years to <12 Years | |
|---|---|---|---|---|
| | **Vol × Frequency** | **Total Vol** | **Vol × Frequency** | **Total Vol** |
| Blood chemistry | 5.0 mL×5 | 25.0 mL | 1.2 mL×5 | 6.0 mL |
| Hematology | 3.0 mL×5 | 15.0 mL | 1.2 mL×5 | 6.0 mL |
| Vitamin B12/iron (including serum pregnancy test in female participants [if applicable]) | 3.5 mL×5 | 17.5 mL | | |
| Blood Phe and Tyr levels | 0.16 mL×23 | 3.68 mL | 0.16 mL×23 | 3.68 mL |
| **Total Blood Volume** | | **61.18 mL** | | **15.68 mL** |

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

## 6.7. Study Intervention Compliance

When the individual dose for a participant is prepared from a bulk supply, the preparation of the dose will be confirmed by a second member of the study site staff.

When participants are dosed at the site, they will receive study intervention directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention. Study site staff will examine each participant's mouth to ensure that the study intervention was ingested.

When participants self-administer study interventions at home, compliance with study intervention will be assessed at each visit. Compliance will be assessed by direct questioning, review of completed dosing diaries, and counting returned study drug during the site visits. Compliance will be documented in the source documents and relevant forms. Deviations from the prescribed dosage regimen will be recorded.

A record of the quantity of sepiapterin and sapropterin dispensed to and administered by each participant must be maintained and reconciled with study intervention and compliance records. Intervention start and stop dates, including dates for intervention delays and/or dose reductions, will also be recorded.

### 6.8. Dose Modification

Dose modification is not permitted in this study.

### 6.9. Continued Access to Study Intervention After the End of the Study

All participants who complete Part 2 will be given the opportunity to continue treatment in the sepiapterin open-label long-term study, PTC923-MD-004-PKU.

### 6.10. Treatment of Overdose

For this study, a dose of sepiapterin or sapropterin >20% than the prescribed daily dose within a 24-hour time period will be considered an overdose.

PTC does not recommend specific treatment for an overdose.

In the event of an overdose, the investigator/treating physician should:

- Report the overdose to PTC Pharmacovigilance only when associated with an SAE.
- Contact the medical monitor immediately.
- Evaluate the participant to determine, in consultation with the medical monitor, whether study intervention should be interrupted or whether the dose should be reduced.
- Closely monitor the participant for any AE/SAE and laboratory abnormalities until resolved or deemed clinically nonsignificant.
- Document the quantity of the excess dose as well as the duration of the overdose.

### 6.11. Concomitant Therapy

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

Any treatments (including nutritional supplements) and over-the-counter medications (including herbal medications) taken by a participant 30 days prior to the Screening Visit and during the course of the study and the reason for use of the medication will be recorded in the source and on the electronic case report form (eCRF). Additionally, specific information related to previous use of pegvaliase-pqpz will be collected (ie, details on duration; dose; if discontinued, the reason[s] why; and if a participant is known to be $BH_4$ responsive). During Screening, each participant will be instructed to report the use of any medication to the investigator. Participants will also be instructed about the importance of not taking any medication throughout the study (including over-the-counter medications) without consulting the investigator.

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

## 6.12. Rescue Medicine

During the study, if blood Phe levels decrease at any time below the lower limit of normal (<35 μmol/L) while on treatment, an unscheduled blood Phe sample should be collected and rescue therapy consisting of extra protein starting at 0.25 g/kg/day given depending on participant preference should be consumed. If hypophenylalaninemia persists for a participant, total daily protein consumption may be increased in 1-g increments every 2 weeks. Any increase in total daily protein consumption will be captured in the study database via 3-day diet records per the schedule of assessments. The participant or participant's parent(s)/legally designated representative(s) will complete a 3-day diet record. If considered necessary by the investigator, additional unscheduled 3-day diet record collection is permitted.

# 7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

## 7.1. Discontinuation From Study Drug Administration

Premature discontinuation of study drug administration is defined as the discontinuation of study drug for an individual participant 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 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 participant's permanent discontinuation from study drug administration
- In the judgment of the investigator, the participant experiences a general or specific change(s) that renders the participant unsuitable for continued study drug administration
- There is a need for concomitant medication that makes the participant ineligible for further study drug administration

Adult participants (≥18 years of age) 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. After completion of these assessments, the participant should revert to their prestudy SoC to treat PKU (at the discretion of the investigator or the participant's physician). Pediatric participants (<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 participant's physician). In addition, a follow-up phone call will occur 30 (±3) days after the last dose of study drug to assess for SAEs.

### 7.1.1. Participant Discontinuation/Withdrawal From the Study

Participants may withdraw from the study for any reason or be withdrawn at the request of the investigator or PTC. The reason for a participant'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 the following:

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

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

## 7.2. Lost to Follow-Up

A participant is considered lost to follow-up if he/she does not complete the study and attempts to contact the participant are unsuccessful. Efforts must be made on the part of the site to avoid any participant being lost to follow-up during the study. Before any participant 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 participant.

# 8. STUDY ASSESSMENTS AND PROCEDURES

## 8.1. Efficacy Assessments

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 after 4 weeks of treatment with sepiapterin versus sapropterin. Using the average over a 4-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 7, Table 8, and Table 9. 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 predose or postdose.

Samples for blood Phe levels measurement will be collected while the participant is either in the clinic or at home, depending on whether the participant has a scheduled clinic visit. Dried blood samples obtained at home will be shipped to the study site or returned to the study site at the next clinic visit. Samples will be collected using the VAMS technology by utilizing the Mitra microsampling devices provided by Trajan (formerly 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 participants, allowing blood samples (20 µL per sample) to be collected at home, without clinical visits, and samples are shipped at room temperature. When participants provide samples in clinic, site staff can provide additional training on the sampling technique if considered necessary or requested. Analysis of the screening blood Phe level can be performed at the site's local laboratory for determination of eligibility; however, a separate

sample for blood Phe/Tyr should still be sent to the central laboratory for the screening timepoint.

**Table 7: Phe/Tyr Sample Collection (Screening Period)**

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

**Abbreviations**: $BH_4$, tetrahydrobiopterin; DBS, dried blood sample; Phe, phenylalanine; Tyr, tyrosine
Note: Analysis of the screening blood Phe level can be performed at the site's local laboratory for determination of eligibility; however, a separate sample for blood Phe/Tyr should still be sent to the central laboratory for the screening timepoint.
If $BH_4$ Washout Period Day 7 is the same day as Day -1 of Part 1, only 1 dried blood sample each for Phe/Tyr should be collected.

**Table 8: Phe/Tyr Sample Collections (Part 1)**

| | Part 1 - Open-Label Responsiveness Test | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Sepiapterin Open-Label Treatment 14 Days | | | | | Sepiapterin Washout Minimum 14 Days/Maximum 21 Days | | |
| Study Day | -1 | 1 | 7 | 10 | 14 | 19 | 24 | 28 |
| DBS | X | X | X | X | X | X | X | X |

**Abbreviations**: DBS, dried blood sample; ETV, Early Termination Visit; Phe, phenylalanine; Tyr, tyrosine
Note: If a participant discontinues the study early, an additional DBS should be taken at the ETV.
Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
Day -1 and Day 1 samples for Part 1 should be taken predose. All other samples can be collected predose or postdose.
A window of ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.
If Part 1 Day 28 (sepiapterin washout) and Part 2 Treatment Period 1 Day -1 are on the same day, only 1 dried blood sample each for Phe/Tyr should be collected.

**Table 9: Phe/Tyr Sample Collections (Part 2) and End of Study or Early Termination Visit**

| | Sepiapterin 60 mg/kg vs Sapropterin 28 Days (4 Weeks) | | | | | | | | Washout 1 14 Days | | | Sepiapterin 60 mg/kg vs Sapropterin 28 Days (4 Weeks) | | | | | | | | Washout 2 14 Days | | | EOS or ETV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Study Day | -1 | 1 | 7 | 10 | 14 | 19 | 24 | 28 | 5 | 10 | 14 | -1 | 1 | 7 | 10 | 14 | 19 | 24 | 28 | 5 | 10 | 14 | |
| DBS | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |

**Abbreviations**: DBS, dried blood sample; EOS, End of Study; ETV, Early Termination Visit; Phe, phenylalanine; Tyr, tyrosine
Note: If a participant discontinues the study early, an additional DBS should be taken at the ETV.
Samples will be collected after fasting or no earlier than 3 hours postprandial at approximately the same time of day at each collection timepoint.
Day -1 and Day 1 samples for Part 2 should be taken predose. All other samples can be collected predose or postdose.
A window of ±1 day is permitted for samples collected after Day 1. For Day -1 and Day 1, samples must be collected on the specified day.
If Washout 1 Day 14 and Treatment Period 2 Day -1 are on the same day, only 1 dried blood sample each for Phe/Tyr should be collected.

## 8.2. Exploratory Efficacy Assessments

### 8.2.1. 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 7, Table 8, and Table 9. 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 participant is either in the clinic or at home, dependent on whether the participant 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 participants, allowing blood samples (20 µL per sample) to be collected at home, without clinic visits, and samples are shipped at room temperature. When participants provide samples in clinic, site staff can provide additional training on the sampling technique if considered necessary or requested.

### 8.2.2. PKU-QOL Questionnaire

In this study, a PKU-specific version of the QOL questionnaire will be used. Four versions of the PKU-QOL will be used: Parent (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, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French. Conduct of the PKU-QOL will not be required for participants whose primary language is not one of the available validated languages.

PKU-QOLs comprises 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; and for other scores, a higher score is associated with a greater impact (Alptekin 2018).

The PKU-QOL questionnaire will be administered to the subset of participants whose primary language is English, Turkish, Dutch, German, Spanish, Italian, Portuguese, or French.

### 8.2.3. 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 participants aged ≥16 years, the EQ-5D-5L will be used. For the EQ-5D-5L, respondents rate their own health on each dimension; each dimension has 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 participant

is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the 5 dimensions. 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 should 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.

## 8.3. Safety Assessments

### 8.3.1. 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; and neurological. 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 participant is known to be $BH_4$ responsive).

A minimum of 2 historical blood Phe values ≥600 µmol/L will be collected (these may include newborn screening blood Phe concentrations, if available). Additionally, the 3 most recent Phe concentrations will be collected.

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

### 8.3.2. 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, participants will rest for 5 minutes in a supine position before vital signs are assessed.

Vital signs will be collected as indicated in Table 1 and Table 2. Vital signs will be collected both predose and 2 hours postdose on Part 1 Day 1, Part 2 TP1 Day 1, and Part 2 TP2 Day 1; for all other timepoints, they will be taken at any time during the visit prior to laboratory sampling.

Weight will be collected as indicated in Table 1 and Table 2. Measurements are to be completed prior to initial dosing for Part 1 Day 1 and Part 2 TP1 Day 1 and Part 2 TP2 Day 1. Weight can be measured at any time during the other visits.

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

Permitted time windows are presented in Table 1 and Table 2.

### 8.3.3. Physical Examination

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

### 8.3.4. Electrocardiogram

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

Permitted time windows are presented in Table 1 and Table 2.

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.

### 8.3.5. Laboratory Assessments

Blood and urine samples for clinical chemistry, hematology, and urinalysis will be collected at the timepoints in Table 1 and Table 2. Participants should be fasted prior to collection of samples (minimum of 4 hours for participants <6 years and minimum of 8 hours for participants ≥6 years prior to sample collection). Pregnancy tests are required for all women of childbearing potential; serum pregnancy testing to occur during the initial Screening Visit and urine pregnancy testing to occur during all other in-clinic visits. Any positive urine pregnancy test should be confirmed by a serum pregnancy test performed at the site's local laboratory.

Laboratory parameters that will be assessed will include but not be limited to the following:

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

Additionally:

- At the EOS Visit, eGFR should be calculated (all participants).
- At any unscheduled visits in which eGFR calculation is desired, serum creatinine should be measured.

### 8.3.6. 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).

### 8.3.7. Diet Monitoring: 3-Day Diet Records

Participants will be instructed to continue their usual diet without modification (ie, no change in total protein, non-Phe protein from medical formula, or daily Phe consumption). Participants 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 and will be collected weekly (during Screening and the Part 1 Treatment Period) and biweekly (during the Part 1 Washout Period and Part 2 Treatment and Washout Periods). At each in-clinic visit, a dietitian will review each participant's diet to monitor and assess protein/Phe consumption and to have regular contact with the participants. The total Phe consumption during the 3-day period will be calculated by the dietitian and entered in the eCRF.

During Screening, dietary Phe intake will be measured by weekly 3-day diet records. Participants who have a >20% variance in dietary Phe consumption during the 24- to 30-day Dietary Control Observation Period will be considered screen failures.

### 8.3.8. *PAH* Genotype Testing

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

## 8.4. Taste, Palatability, and Acceptability Assessment

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

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

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

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

## 8.5. Adverse Events, Serious Adverse Events, and Other Safety Reporting

### 8.5.1. Definition of Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not it is considered related to the drug. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease in a study participant 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 8.5.2, any hospitalization occurring as the consequence of an AE during the study period should be reported as an SAE.

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

### 8.5.2. Definition of Serious Adverse Events

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

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

### 8.5.3. Unexpected Adverse Events

The Investigator's Brochure contains the 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.

### 8.5.4. Eliciting Adverse Event Information

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

### 8.5.5. Recording Non-Serious Adverse Events and Serious Adverse Events

All AEs (both serious and non-serious) that occur in participants 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 MedDRA should be employed. In addition, the following information should be recorded:

- Indication of whether the event is serious or non-serious (Section 8.5.2)
- Relationship to study drug (Section 8.5.6)
- Severity of the event (Section 0)
- 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.

### 8.5.6. Describing Adverse Event Relationship to Study Drug

The investigator should provide an assessment of the relationship of the AE to the study drug, ie, whether there is a reasonable possibility that the study drug caused the AE, using the considerations outlined in Table 10.

**Table 10: Relationship of Study Drug to AE**

| 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 participant's disease state, other medical conditions, or environmental factors. |
| 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 participant's disease state, other medical conditions, or environmental factors. |

**Abbreviations**: AE, adverse event

### 8.5.7. 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. 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 11.

**Table 11: 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 participant's overall health and well-being, does not interfere with the participant'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 |

### 8.5.8. Adverse Event Reporting

Investigator site reporting requirements for AEs are summarized in Table 12.

**Table 12: 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 participant 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 participant to discontinue the study.

Intensity will be assessed according to the scaling demonstrated in Table 11.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity (Section 0), whereas seriousness is defined by the criteria under Section 8.5.2. An AE of severe intensity may not be considered serious.

Should a pregnancy occur in a participant or a female partner of a participant, 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 participant 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 participant 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 participant 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).

### 8.5.9. 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 the EDC system.

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

Follow-up information to the SAE should be clearly documented 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 participant's name, address, and other personal identity information are obscured. Only the participant'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 participant 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: Pharmacovigilance@ptcbio.com, Facsimile: 1 (908) 325-0355**

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

### 8.5.10. Contraception

A woman is considered of childbearing potential, ie, fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. 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 participant) 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 study 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).

### 8.5.11. Reporting Pregnancy

PTC should be notified in the event that a female participant in the study or a female partner of a male participant 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. 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 participant in the study.

In any of the four situations listed below, the participant 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.

- Participant becomes pregnant while participating in the study.
- Female partner of a male participant participating in the study becomes pregnant.
- Participant becomes pregnant up to 90 days after the last administration of study drug (60 days after the 30-day safety follow-up telephone call).
- Female partner of a male participant 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 participant should be asked to sign this consent form. However, signing this form is voluntary; it is up to the participants to decide whether to agree to the collection of this information or not. Upon signing, the participant'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 participant or the pregnant female partner of a male participant 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.

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 Therapeutics 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 participant's identity and the identity of the child. In particular, the participant'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 participant participating in the study does not wish to provide such information, this will not prevent the partner from continuing with the study.

### 8.6. 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 (SUSARs) 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 an 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. Serious adverse events 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.

## 8.7. PTC Expedited Reporting of SUSARs to EudraVigilance

In the EU, as per requirements of EU Clinical Trials Regulation No. 536/2014 of the European Parliament and of the Council of 16 April 2014 on Clinical Trials on Medicinal Products for Human Use, and Repealing Directive 2001/20/EC, SUSARs should be submitted to the EudraVigilance database. Additional direct submissions from the sponsor to Ethics Committees and National Competent Authorities are not required.

As per the EU Clinical Trials Regulation, the sponsor shall notify the Member States concerned of all findings that affect the benefit-risk balance of the medicinal product or clinical trial and any urgent safety measures (USMs) taken. The sponsor will submit findings that affect the benefit-risk balance or any USM via the Clinical Trials Information System.

# 9. STATISTICAL CONSIDERATIONS

A statistical analysis plan (SAP) will be prepared and approved prior to study database lock 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 blood Phe levels from baseline to Weeks 3 and 4 (ie, the average of the last 2 weeks of each treatment period in Part 2) is the same between sepiapterin and sapropterin versus the alternative that they are different; a 2-sided test at the 5% alpha level will be applied.

## 9.2. Sample Size Determination

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

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

## 9.3. Analysis Sets

Four study populations will be analyzed:

- Primary Analysis Set (PAS): All participants who achieved a ≥30% reduction in blood Phe concentrations in Part 1, are randomized, and take at least 1 dose of study drug in Part 2 will be included in the PAS. Participants will be analyzed according to their randomized treatment.
- Full Analysis Set (FAS): All participants who are randomized and take at least 1 dose of study drug in Part 2 will be included in the FAS. Participants 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 participants 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 detailed in the SAP.
- Safety Analysis Set: All participants who receive at least 1 dose of study drug, including during Part 1, will be included in the Safety Analysis Set. Participants will be analyzed according to actual treatment received.

## 9.4. Statistical Analyses

### 9.4.1. General Considerations

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

### 9.4.2. Analysis of Efficacy Endpoints

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

For the primary efficacy endpoint, a gatekeeping procedure will be used to control the familywise error rate.

The stratum of participants with mean % reduction in Phe levels of ≥30% during Part 1 (PAS) will be tested at the significance level of 0.05 (2-sided). If $p<0.05$, then the study will be declared positive; otherwise the study would be declared negative.

Only if the test based on the PAS is statistically significant at the 0.05 level will the FAS be tested, also at the 0.05 significance level.

The baseline for Part 1 is the average of Day -1 and Day 1 predose blood Phe concentration values.

For Part 2 Treatment Period 1, the baseline is the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 1. For Part 2 Treatment Period 2, baseline is the average of Day -1 and Day 1 predose blood Phe concentration of Part 2 Treatment Period 2.

A mixed model repeated measures (MMRM) model will be fitted on the calculated mean change in blood Phe from baseline to each of the 2-week postbaseline assessment intervals within the treatment period (Periods 1 and 2) for each participant. The model will include fixed effects for treatment group, sequence, period, % Phe reduction from Part 1 (≥20% to <30% or ≥30%), visit (Weeks 1 to 2 and Weeks 3 to 4), and visit-by-treatment interaction, and the baseline blood Phe (for each period) as a covariate. In addition, participant nested within sequence will be included as a random effect. The least squares (LS) mean estimate for the change in blood Phe levels from baseline to average of Weeks 3 and 4 will be used to perform treatment group comparisons. The LS means, treatment effect estimate, 95% CI, and 2-sided p value will be presented.

For the primary analysis, MMRM model will be used on the available data assuming the missing assessments are missing at random ; no explicit imputation is involved. The following sensitivity analyses will be performed:

1. Completer analysis with analysis of covariance model: only the completers who have the assessments at Weeks 3 and 4 in each period of Part 2 will be included in the model.
2. Multiple imputation (MI): the MI procedure will assume the missing data are missing not at random. The procedure will be applied by each period if there is at least 10% missing assessments (ie, ≥2 participants) at Weeks 3 and 4 at that period in Part 2.

Further details are provided in the SAP regarding sensitivity analysis on missing data handling in the study.

### 9.4.3. Safety Analyses

#### *9.4.3.1. Participant Disposition*

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

#### *9.4.3.2. Medical History and Prior Medication*

Medical history and prior medication information will be descriptively summarized.

#### *9.4.3.3. 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.

### 9.4.4. Baseline Descriptive Statistics

Demographic and baseline characteristics of participants 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 participants.

#### *9.4.4.1. Laboratory Parameters*

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

#### *9.4.4.2. Adverse Events*

Summary information (the number and percentage of participants 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 participants experiencing AEs divided by the total number of participants.

### 9.4.5. Taste, Palatability, and Acceptability Analyses

Taste, palatability, and acceptability evaluation results will be tabulated for each question.

Additional summaries will be performed as deemed necessary upon review of the data.

## 9.5. Interim Analysis

No formal interim analysis is planned.

# 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 participants in this study. The names and identities of all research participants 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 participant 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 participants 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 participants. 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/she can enroll any participant 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 participants for the study. The protocol must be re-approved by the IRB/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/IEC according to local regulations and guidelines.

## 12.2. Ethical Conduct of the Study and Regulatory Compliance

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. Furthermore, this study will be conducted in compliance with this protocol, EU Regulation No. 536/2014, and the principles of GCP.

## 12.3. Informed Consent Process

By signing the protocol, the investigator assures that informed consent or assent (as required) will be obtained from each participant or legally designated 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 participant or legally designated 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 participant or legally designated representative in a language in which the participant is fluent, or translated, according to local regulations. This information must be provided to the participant or legally designated representative prior to undertaking any study-related procedure. Adequate time should be provided for the participant or legally designated representative to read the informed consent, to understand the risks and benefits of participating in the study, and to ask any questions that the participant may have about the study. The participant or legally designated representative should be able to ask additional questions as and when needed during the conduct of the study. The participant or legally designated representative's signature on the ICF should be obtained at the investigator site in the presence of the investigator or a qualified representative (eg, subinvestigator).

Each participant 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 participants (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 system for each participant (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, participant 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 25 years (or longer depending on local requirements) after completion or discontinuation of the study 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. The investigator shall keep medical records based upon the national law or for at least 15 years.

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 participant, investigator, or site staff. Examples of deviations include but are not limited to the following:

- 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, medical history, etc) - either tests are not done, incorrect tests are done, or tests are 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 participant eligibility (ie, protocol inclusion/exclusion violations), participant safety, or a participant's ability to continue in the clinical study.

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 study 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 PTC.

PTC 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/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: 13 July 2023

Version 2.0: 20 March 2024

Version 3.0: 11 April 2024

Version 4.0: 06 September 2024

## 15.1. Version 4.0: 06 September 2024

The overall reason for Version 4.0 of the protocol was to incorporate Health Authority feedback.

| **Protocol Section** | **Version 4.0/ Description of Change** | **Reason/ Rationale** |
|---|---|---|
| Throughout | Updated version number and date; minor editorial and document formatting revisions | Minor updates; therefore, they have not been summarized |
| Throughout | "Dietary Observation Period" updated to "Dietary Control Observation Period" | Updated for consistency |
| Synopsis; Schedule of Assessments Table 1 Footnote o;<br>Schedule of Activities Table 2 Footnote p; | To detail expected DBS sampling if $BH_4$ Washout Period Day 7 occurs on the same day as Part 1 Day -1 and if Part 2 Washout Period 1 Day 14 occurs on the same day as Part 2 Treatment Period Day -1. | To eliminate duplicate DBS sampling for visits that are permitted to occur on the same day |
| Section 4.1.2.2;<br>Section 4.1.3;<br>Section 5<br>Section 8.1 Footnotes on Tables 7, 8, and 9 | Added detail that biochemically diagnosed classical PKU population will be capped at 30% in the Safety Analysis Set. | To specify precisely which 'study population' is being referred to |
| Section 1.2 (Schedule of Activities) | A +1-day window has been added for virtual visits in Table 1 and Table 2. | Added to allow for visits that occur on a weekend |
| Section 2.6.2.2 | Explanation for the selection of the PAS was added. | Detailed explanation of PAS, per CTIS feedback |
| Section 6.3 | Added acceptable methods for disposing of (destroying) study drug. | Acceptable method for the destruction of study drug is by incineration. |
| Section 6.6 | Part 1, consisting of the open-label sepiapterin responsiveness test and sepiapterin washout period, may last up to 35 days. | Clarification regarding duration of total responsiveness and washout period |
| Section 6.11 | Updated language regarding childhood vaccination and COVID-19 vaccination | To add specific language to allow for COVID-19 and other routine vaccinations |
| Section 8.3.7 | Updated to state that the Dietary Control Observation Period is 24 to 30 days. | To specify the acceptable range for the 4-week Dietary Control Observation Period |

## 15.2. Version 3.0: 11 April 2024

The overall reason for Version 3.0 of the protocol was to incorporate Health Authority feedback.

| Protocol Section | Version 3.0/Update | Reason/ Rationale |
|---|---|---|
| Protocol | The version number and date were updated throughout.<br>Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity.<br>The synopsis, study schema, and Schedule of Assessments were updated to be consistent with changes in the protocol. | Update |
| Throughout | Clarification that the maximum washout period for Part 1 is 21 days. | Update |
| | Clarification that each washout period in Part 2 consists of 14 days (+3-day window).<br>Clarification of the min and max duration of the dietary control observation period. | Update |
| Section 5.1 | Inclusion criterion 1 was updated to remove reference to “incapacitated adults.” | Update |
| Section 5.2 | Exclusion criterion 1 was updated to specifically exclude “incapacitated adults.” | Update |
| Section 9.3 | Definition of Primary Analysis Set was added. | Update |
| Section 9.4.2 | Additional details were added to clarify analysis of efficacy endpoints. The definition for baseline blood Phe for each part and period was also clarified. | Update |

## 15.3. Version 2.0: 20 March 2024

The overall reason for Version 2.0 of the protocol was to incorporate Health Authority feedback.

| Protocol Section | Version 2.0/Update | Reason/ Rationale |
|---|---|---|
| Protocol | The version number and date were updated throughout.<br>Editorial and administrative revisions (eg, typographical errors, punctuation, tenses, abbreviations) were incorporated to provide clarity.<br>The synopsis, study schema, and Schedule of Assessments were updated to be consistent with changes in the protocol. | Update |
| Throughout | The term “dietician” was replaced with “dietitian” throughout. | Update |
| | The term “sapropterin dihydrochloride” was replaced with “sapropterin” throughout. | Update |
| | The term “legal guardian” was replaced with “legally designated representative” | Update |
| | “Follow-up phone call” legend was updated to include “discontinue the study prematurely.” | Update |
| Table 1 | Eligibility check was added to Day 1. | Update |
| | Blood Phe/Tyr sampling was added to ETV. | Update |
| | Footnotes d and i were updated to remove “skin” as an assessment parameter. | Update |

| Protocol Section | Version 2.0/Update | Reason/ Rationale |
|---|---|---|
| | Footnote q was updated to specify 3-day diet record maintenance timepoints. | Update |
| | Table note was updated to change window on ETV. | Update |
| | Table note was updated to add the option to return study drug during the next in-clinic visit. | Update |
| Table 2 | Consistent diet/diet monitoring/3-day diet record row was formatted to clearly show that diet records are collected during Part 2 TP2 Washout. | Update |
| | Collect study drug, assess compliance row was updated to remove P2 WO2, P2 TP1 D14, and P2 TP2 D14 timepoints. | Update |
| | Footnote j was updated to remove “skin” as an assessment parameter. | Update |
| | Footnote o was updated to include ±3-day window for follow-up phone call. | Update |
| | Footnote p was updated to detail that if Washout Period 1 Day 14 is the same day as Day -1 of TP2, only 1 dried blood sample should be collected. | Update |
| | Footnote q was updated to 3-day diet records should be collected biweekly during Part 2 Treatment and Washout Periods. | Update |
| | Table note was updated to add the option to return study drug during the next in-clinic visit. | Update |
| Section 2.6 | Additional subsections (2.6.1 and 2.6.2) were added to provide additional benefit-risk information. | Update |
| Section 3 | Primary endpoint was reworded for clarity. | Update |
| Section 4.1 | Additional detail was added regarding 3-day diet record collection timepoints. | Update |
| Section 4.1.4 | Additional detail was added regarding the EOS Visit timepoint. | Update |
| Section 4.2 | The number and location of study centers were added. The term “enrolled” was changed to “screened” for accuracy. | Update |
| Section 5.1 | Inclusion criterion 1 was updated to provide further detail around incapacitated adults. | Update |
| | Inclusion criterion 3 was updated to remove the word “uncontrolled.” | Update |
| | Inclusion criterion 5 was updated for consistency with CTFG. | Update |
| Section 5.2 | Exclusion criterion 5 was updated to include ingredients and excipients. | Update |
| Section 5.3 | Restriction around consumption of substrates and inhibitor of BCRP transporters was removed. | Update |
| Section 6.7 | “Review of completed dosing diaries” was added as a measurement of compliance. | Update |
| Table 9 | ETV was added to the EOS column. | Update |
| Section 8.3.1 | “Skin” was removed as an assessment parameter. | Update |
| Section 8.3.3 | “Skin” was removed as an assessment parameter. | Update |
| Section 8.3.7 | Additional detail was added regarding 3-day diet record collection timepoints. | Update |
| Section 8.7 | A new section was added to provide details for expedited reporting of SUSARs. | Update |

| Protocol Section | Version 2.0/Update | Reason/ Rationale |
|---|---|---|
| Section 9.2 | The term “enrolled” was changed to “screened” for accuracy. | Update |
| Section 9.4.2 | Additional detail around the analysis methods was added. | Update |
| Section 12.2 | Additional statement around compliance with EU Regulation No. 536/2014 was added. | Update |
| Section 13.2 | Record retention periods were updated. | Update |

## 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 Package Insert.

Bratkovic, D, Margvelashvili, L, Tchan, M, Nisbet, J and 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(15516).

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.

Burton, BK, Grange, DK, Milanowski, A, Vockley, G, Feillet, F, Crombez, EA, et al. The Response of Patients With Phenylketonuria and Elevated Serum Phenylalanine to Treatment With Oral Sapropterin Dihydrochloride (6R-tetrahydrobiopterin): A Phase II, Multicentre, Open-Label, Screening Study. J Inherit Metab Dis 2007;30(5):700-707.

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 Genetics 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 Related 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.

Gao, L, Smith, N, Kaushik, D, Milner, S and Kong, R. Validation and Application of Volumetric Absorptive Microsampling (VAMS) Dried Blood Method for Phenylalanine Measurement in Patients with Phenylketonuria. Clin Biochem 2023;116:65-74.

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 Genetics 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 Genetics 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 SmPC (2020). Kuvan Summary of Product Characterisitcs.

KUVAN USPI (2021). KUVAN United States Prescribing Information. **2022**.

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 and Böhme, E. 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.

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

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 Genetics Metab 2019;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 Genetics 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 and Levy, H. Phenylalanine Blood Levels and Clinical Outcomes in Phenylketonuria: A Systematic Literature Review and Meta-Analysis. Mol Genetics 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.

Signature Page for PTC923-PKU-301 Clinical Protocol V4.0 - 06SEP2024 v5.0

| | |
|---|---|
| Clinical Approval | [redacted]ument(s)<br>07-Sep-2024 14:38:45 GMT+0000 |

| | |
|---|---|
| Clinical Approval | [redacted]ument(s)<br>09-Sep-2024 14:02:39 GMT+0000 |

| | |
|---|---|
| Statistics Approval | [redacted]ment(s)<br>09-Sep-2024 21:00:33 GMT+0000 |

Signature Page for VV-CLIN-007931 v5.0