NCT #NCT03519711

A Phase 1/2, Open-Label, Randomized Parallel Arm, Intra-patient Dose Escalation Study to Evaluate the Safety, Pharmacokinetics and Preliminary Efficacy of CNSA-001 (Sepiapterin) in Primary Tetrahydrobiopterin Deficient Patients with Hyperphenylalaninemia

Protocol: PBD-001

Version 2.0: 09 Nov 2020

STATISTICAL ANALYSIS PLAN

Censa Pharmaceuticals, Inc. was acquired by and is a subsidiary of PTC Therapeutics, Inc. and its name officially changed to PTC Therapeutics MP, Inc. Any reference to Censa Pharmaceuticals, Inc, Censa, PTC Therapeutics MP, Inc. equate to the same organization.

STATISTICAL ANALYSIS PLAN APPROVAL
Protocol: PBD-001
Version 1.0

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09-Nov-2020
Date

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09-Nov-2020
Date

PTC Therapeutics, Inc.

SAP Amendments before database lock

Version	Issue Date	Section	Revision / Addition	Rationale
2	09-Nov-2020	7.4 Medical History	Changed MedDRA from Version 21.1 to Version 23.1	Coding will be done using Version 23.1
2	09-Nov-2020	7.5 Prior and Concomitant Medications	Change WHODrug from Version March 2018 to September 2020	Coding will be done using Version September 2020
2	09-Nov-2020	7.8.1 Adverse Events	Changed MedDRA from Version 21.1 to Version 23.1	Coding will be done using Version 23.1

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LIST OF ABBREVIATIONS

5-HTP	5-Hydroxytryptophan
AE	Adverse event
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	Area under the concentration-time curve
AUC_{0-t}	Area under the concentration-time curve from time zero to time 't' where t is a defined time point after administration
BH_4	Tetrahydrobiopterin
BLQ	Below the limit of quantitation
BMI	Body mass index
bpm	Beats per minute
CANTAB	Cambridge Neuropsychological Test Automated Battery
CASC	Child and Adolescent Sleep Checklist
C_{max}	Maximum plasma drug concentration
CSF	Cerebrospinal fluid
CSR	Clinical study report
DBP	Diastolic blood pressure
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case report form
ECG	Electrocardiogram
EOS	End of Study
FDA	Food and Drug Administration
GGT	Gamma glutamyl transferase
GM	Geometric mean
GCV%	Geometric coefficient of variation %
GTP-CH	Guanosine triphosphate cyclohydrolase I
HR	Heart rate
MedDRA	Medical dictionary for regulatory activities
NIH	National Institutes of Health
PBD	Primary tetrahydrobiopterin deficiency
Phe	Phenylalanine
PD	Pharmacodynamic

PK	Pharmacokinetic
PT	Preferred Term
PTPS	Pyruvoyl-tetrahydropterin synthase
QTc	Corrected QT interval
QTcF	Corrected QT interval calculated using Fridericia's formula
RR	Respiratory rate
RTI	Reaction Time (CANTAB)
RVP	Rapid Visual Information Processing (CANTAB)
SAE	Serious AE
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SOC	System Organ Class
SSP	Spatial Span (CANTAB)
SWM	Spatial Working Memory (CANTAB)
TEAE	Treatment-emergent AE
T_{last}	Time of last observed quantifiable concentration
T_{max}	Time to maximum observed plasma concentration
Tyr	Tyrosine
US	United States
WHO	World health organization

1.0 INTRODUCTION

This Statistical Analysis Plan (SAP) provides the framework for the summarization and analysis of the clinical data from the study, "A Phase 1/2, Open-Label, Randomized Parallel Arm, Intra-patient Dose Escalation Study to Evaluate the Safety, Pharmacokinetics and Preliminary Efficacy of CNSA-001 (Sepiapterin) in Primary Tetrahydrobiopterin Deficient Patients with Hyperphenylalaninemia". Major changes made to the SAP after it has been signed but prior to database lock will be documented in an amendment. Any major changes made to the planned analysis will be described in the clinical study report (CSR).

2.0 STUDY DESIGN

This is a Phase 1/2, multicenter, randomized, open-label, intra-patient dose escalation study designed to evaluate the safety, pharmacokinetics, and preliminary efficacy of CNSA-001 (sepiapterin) in male and female patients with primary tetrahydrobiopterin deficient (PBD).

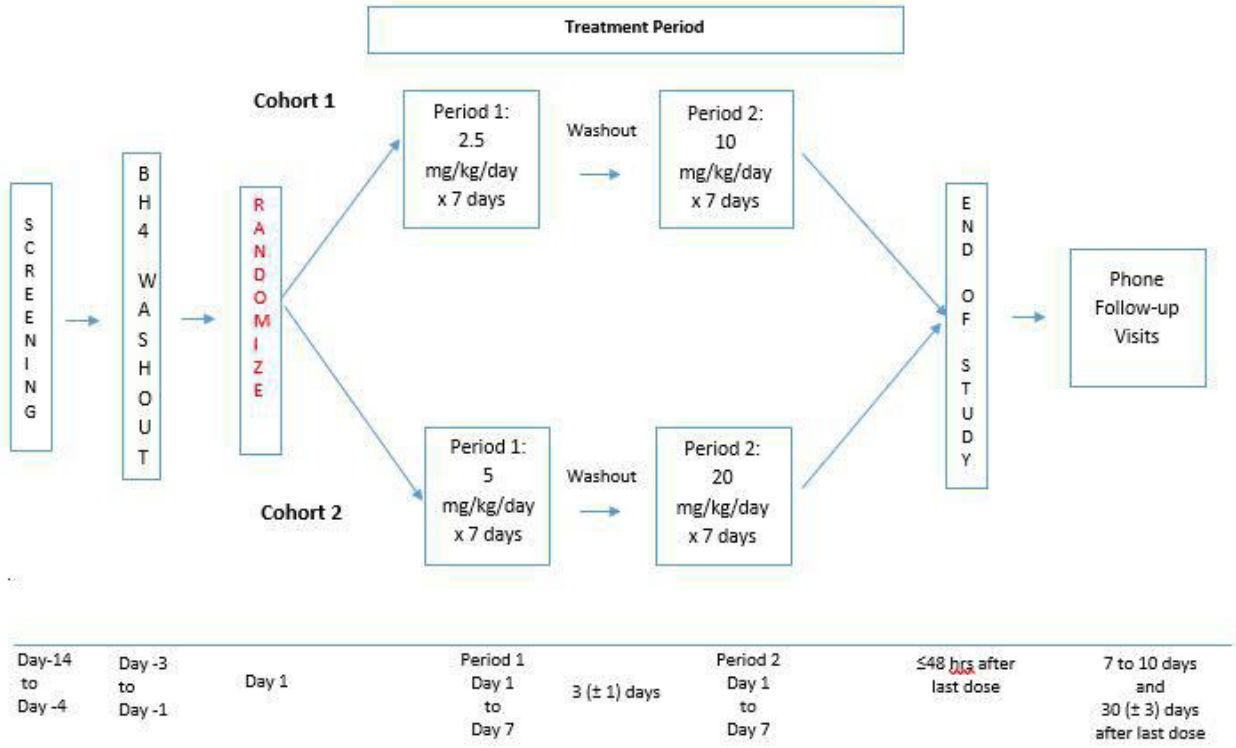
This study will enroll patients with confirmed defects in de novo biopterin biosynthesis due to 6-pyruvoyl-tetrahydropterin synthase (PTPS) or recessive guanosine triphosphate cyclohydrolase I (GTP-CH) genes, abnormal enzyme activity of the PTPS or GTP-CH enzymes, or a cerebrospinal fluid (CSF) biochemical profile indicative of PTPS or GTP-CH deficiencies.

Six (6) to 10 patients will be enrolled in this study at 5 to 6 study centers.

The study design is summarized in the study schema that follows and the schedule of assessments and procedures is provided in Appendix A.

Screening Period (Day -14 to Day -4): An informed consent or assent (if applicable) form must be signed before any study-related procedures are performed. After consenting to the study, PBD patients will undergo Screening procedures which include medical/surgical history, demographics, vital signs, 12-lead electrocardiogram (ECG), physical examination, and clinical laboratory tests (chemistry, hematology, urinalysis). Blood phenylalanine (Phe) levels will be measured at Screening and compared to the 3 most recent historical Phe concentrations. Patients who are eligible based on Screening tests will proceed to the tetrahydrobiopterin (BH_4) Washout Period.

BH_4 Washout Period (Day -3 to Day -1): Eligible patients who are taking BH_4 [Kuvan® (sapropterin dihydrochloride)] will discontinue the medication during the BH_4 Washout Period and will remain off this medication during the entire study. Patients will be instructed to maintain a consistent diet (with respect to protein and Phe intake) and 3-day diet records will be collected during BH_4 Washout Period and throughout the study. On Days -3, and -1 during the BH_4 Washout Period, blood will be collected for determination of Phe concentrations.



Treatment Period 1 and Period 2: Patients will receive treatment with CNSA-001 twice daily for a total of 14 days (that is, two 7-day treatment periods separated by a 3 (±1) day washout). Patients will be randomized into one of 2 cohorts, with each cohort assessing 2 dose levels of CNSA-001 via intra-patient dose escalation.

- **Cohort 1**, patients will receive 2.5 mg/kg/day for 7 days in Period 1, undergo a 3 (±1) day washout period, then escalate to 10 mg/kg/day for 7 days in Period 2 (14 days total treatment)
- **Cohort 2**, patients will receive 5 mg/kg/day for 7 days in Period 1, undergo a 3 (±1) day washout period, then escalate to 20 mg/kg/day for 7 days in Period 2 (14 days total treatment).

Patients will be eligible for dose escalation during Period 2 if they meet the criteria for intra-patient dose escalation (refer to Section 6.12 of protocol).

Data Safety Monitoring Board (DSMB) and possible inclusion of patients between 12 months and 17 years of age inclusive: Initially, only adult patient(s) (≥18 years) will be enrolled. The first adult patient will be enrolled into Cohort 1 and then a second adult patient will be enrolled into Cohort 2 of the study, with subsequent patients being randomized into either cohort. After the first adult patient(s) have completed the study, the DSMB will review safety and

pharmacokinetic/ pharmacodynamic (PK/PD) data, including preliminary efficacy, for the adult patient(s). If the data display no safety issues and provide for the prospect of clinical benefit in patients ≥ 12 months to < 18 years old, then the eligibility criterion for age at time of enrollment will be expanded to include children (≥12 months). The safety, and PK/PD data on the dosed adult patient(s) will be submitted for review to the United States (US) Food and Drug Administration (FDA) for review prior to enrolling patients ≥12 months to < 18 years old.

Acceptable Concomitant Medications: During the study, patients will continue their other current medications for PBD (including L-dopa/carbidopa, 5-Hydroxytryptophan (5-HTP), melatonin, Monoamine oxidase inhibitors, and dopamine receptor agonists as prescribed) except for BH_4 supplementation (if they were taking BH_4), and will be monitored clinically as per standard of care for PBD to optimize treatment.

Safety and tolerability will primarily be assessed by adverse events (AEs), vital signs, clinical laboratory tests including chemistry, hematology, and urinalysis, physical examinations, and 12-lead ECGs. Preliminary efficacy will be assessed by the reduction in plasma Phe levels. Other secondary measures will include whole blood serotonin, serum prolactin and BH_4, and urine sepiapterin, BH_4 and neopterin.

Blood samples will be collected to characterize the pharmacokinetics of sepiapterin and its effect on serum BH_4, Phe, and tyrosine (Tyr) levels.

Discontinuation of Study Drug: Patients will receive treatment with CNSA-001 for a total of 14 days (that is, two 7-day treatment periods separated by a 3 (±1) day washout), unless they meet criteria for discontinuing CNSA-001 treatment. Permanent discontinuation of CNSA-001 treatment may be triggered by safety reasons or lack of efficacy. Patients who discontinue CNSA-001 treatment early will complete the End of Study (EOS) assessments. After completion of the EOS assessments, patients should immediately revert to pre-study standard of care for their PBD, including BH_4 if they were taking it previously.

Phone follow-up visits to assess for AEs and serious AEs (SAEs) will occur 7 to 10 days, and 30 (±3) days after the last dose of study drug.

3.0 STUDY OBJECTIVES

Primary:

- To assess the safety and tolerability of 4 dose levels of CNSA-001 in PBD patients with PTPS or recessive GTP-CH deficiency.

Secondary:

- To assess the PK profile of CNSA-001 and its effect BH_4, Phe, and Tyr in PBD patients with PTPS or recessive GTP-CH deficiency.
- To evaluate the preliminary efficacy of CNSA-001 in reducing blood Phe levels in PBD patients with PTPS or recessive GTP-CH deficiency after 7 days of treatment.

Exploratory:

- To assess the change from baseline in other exploratory biomarkers of this disease such as serum prolactin, whole blood serotonin, urine sepiapterin, BH_4, and neopterin levels.
- To assess the change from baseline in Cambridge Neuropsychological Test Automated Battery (CANTAB) tests, including the Reaction Time (RTI), the Spatial Span (SSP), the Spatial Working Memory (SWM) and Rapid Visual Information Processing (RVP).
- To assess changes in movement and sleep for euphenylalaninemic and hyperphenylalaninemic states as assessed by an accelerometer device (GeneActiv, Activinsights Ltd.).
- To evaluate changes from baseline in cognitive assessments via the National Institutes of Health (NIH) Cognition Toolbox.
- To evaluate changes from baseline in the Child and Adolescent Sleep Checklist (CASC).

4.0 ANALYSIS POPULATIONS

4.1 Safety Population

The Safety population will consist of all randomized patients who receive any amount of study drug. All safety analyses will be performed on this population. Any patients who receive the wrong dosing of study drug for their entire course of treatment will be analyzed in the dosing group based on the drug dosing received.

4.2 Efficacy Population

The Efficacy population will consist of all patients who were randomized, received any amount of study drug, and had available pre-dose Phe concentrations at Day 1 and at least one post-Day 1 visit within a given period. All efficacy analyses will be performed on this population.

4.3 PK Population

The PK population will consist of all patients who received at least one dose of study drug and had at least 1 quantifiable post-dose blood sample collected for analysis of sepiapterin, BH4, Phe, or Tyr concentrations. All PK analyses will be performed on this population.

5.0 DEFINITIONS OF OUTCOME MEASURES

Efficacy will be assessed in the Efficacy population. Efficacy measurements considered, are as follows:

5.1 Efficacy Outcomes

Outcome	Measure-ment type
Day 7 change in plasma Phe levels with respect to baseline	Continuous
Number and percent of patients with Day 7 Phe concentrations in acceptable treatment range of 130 to 360 µmol/L	Number and Percent
Number and percent of patients with Day 7 Phe concentrations <130 µmol/L	Number and Percent
Biomarker measurements at each visit, from baseline onwards as per the schedule of assessments (Appendix A): • Biomarkers in urine sepiapterin, neopterin, and BH4 • Serum prolactin • Whole blood serotonin	Continuous
CANTAB at Screening, Day 1 (pre-dose) and Day 7 of each treatment period	Continuous
Movement and sleep assessment via the GENEActiv accelerometer	Continuous
NIH Toolbox Cognitive Domain test battery	Continuous
Parent or caregiver assessment of sleep (CASC)	Categorical

5.2 Safety Outcomes

Safety will be assessed by analysis of the occurrence of treatment emergent AEs (TEAEs), as well as by adverse changes in laboratory evaluations (hematology, serum chemistry panel, urinalysis, and urine microscopic), ECG parameters, vital signs, and physical examinations.

Laboratory abnormalities will not be considered AEs unless the abnormality is part of a clinical signs or syndrome. However, a clinically significant laboratory abnormality (e.g. detected on hematology, serum chemistry panel, urinalysis, and urine microscopic evaluations) that is independent from the underlying medical condition and that requires medical or surgical intervention, or leads to study drug interruption or discontinuation, will be considered an AE.

6.0 STATISTICAL METHODS AND GENERAL CONSIDERATIONS

6.1 Sample Size

The primary objective of this study is to evaluate the safety of 4 doses of CNSA-001. The sample size for this study is not based on statistical considerations, and a sample size of 6 to 10 patients (with 2 doses administered per patient) is considered adequate for this orphan population. Also, the treatment induced changes in Phe level is not powered for this study and caution is needed in the interpretation of efficacy results.

6.2 Randomization

This is an open-label randomized parallel arm, intra-patient dose escalation study. Initially, only adult patient(s) (≥18 years) will be enrolled. The first adult patient will be enrolled into Cohort 1 and then a second adult patient will be enrolled into Cohort 2 of the study, with subsequent patients being randomized into either cohort. After the first adult patient(s) have completed the study, the DSMB will review safety and PK/PD data, including preliminary efficacy, for the adult patient(s). If the data display no safety issues and provide for the prospect of clinical benefit in patients ≥12 months to <18 years old, then the eligibility criterion for age at time of enrollment will be expanded to include children (≥12 months).

6.3 Interim Analysis / DSMB

An interim analysis to assess efficacy is not planned. However, a DSMB will review safety and PK/PD data. A detailed DSMB charter will outline the responsibilities, membership, format and frequency of the meetings, methods of providing data to and from the DSMB, statistical issues, and communication pathways. At a minimum, the DSMB will consist of a physician familiar with PBD, a statistician, and the Medical Monitor.

6.4 Standard Calculations

Variables requiring calculation will be derived using the following formulas:

- Baseline: A baseline value, unless specified otherwise, is the last non-missing value recorded prior to the first dose of study drug for a given dosing period. If an assessment has both a date and time that exactly match the date and time of first dose of study drug, the assessment will be counted as baseline. Note each dosing period within a cohort will have its own baseline.
- Change from Baseline: Change from baseline will be calculated for each patient at the specified time point as the value at the specified time point minus the baseline value.

- Study day: For a given date (date), study day is calculated as days since the date of first dose of study drug within the dosing cohort (firstdose). For example, if the washout between treatment periods is 3 days long, then period 2 day 1 will become study day 11:

  Study day = date – firstdose + 1, where date ≥ firstdose
  Study day = date – firstdose, where date < firstdose
- Days: Durations, expressed in days, between one date (date1) and another later date (date2) are calculated using the following formula: duration in days = (date2 – date1 + 1).
- Weeks: Durations, expressed in weeks, between one date (date1) and another later date (date2) are calculated using the following formula: duration in weeks = (date2 – date1 + 1) / 7.
- Months: Durations, expressed in months, between one date (date1) and another later date (date2) are calculated using the following formula: duration in months = (date2 – date1 + 1) / 30.4375.
- Years: Durations, expressed in years, between one date (date1) and another later date (date2) are calculated using the following formula: duration in years = (date2 – date1 + 1) / 365.25.
- Body Mass Index (BMI): BMI (kg/m^2) = weight (kg) / [(height (cm)/100)^2]
- Age at time of consent is collected.

6.5 Comments on the Statistical Analyses

- All relevant data captured on the electronic Case Report Forms (eCRFs), including specific descriptions of 'other' and comments fields will be included on the listings. All listings will be sorted by patient number in ascending order within dose cohort and period.

- All summary tables will be presented by dose cohort and dose / period. Disposition, discontinuations, demographic and other baseline characteristics, medical history, AEs, and prior and concomitant medication summaries will also include an overall summary column. Summary tables presenting results by study visit will include all scheduled study visits using informative visit labels.

- Continuous variables will be summarized using number (n), mean, standard deviation (SD), median, minimum, and maximum. Except for the time to maximum observed plasma concentration (T_{max}), summaries of PK parameters will also include the geometric mean (GM) and geometric coefficient of variation percentage (GCV%).

- Categorical variables will be summarized by presenting the frequency and percent. Percentages will be based on the patient population unless otherwise specified. For each variable, all categories will be shown. Zero frequencies within a category will be presented without percent.

- If a laboratory result is reported relative to a lower/upper range of detection for an assay (e.g. "<10") the numeric portion of the result (e.g. 10) will be used for statistical analyses and the full result, including any symbols, will be provided in the patient listings.

- Version 9.4 (or higher) of SAS statistical software package will be used to provide all summaries, listings, figures and statistical analyses. R version 4.0.2 using PKNCA package version 0.9.4 will be used to calculate the PK parameters.

6.6 Decimal Places

- For PK data, individual concentrations and PK parameters will be reported to 3 significant figures. For summary statistics, n will be reported as a whole number; mean, SD, median, min, max, geometric mean, CV% and GM CV% will be reported to 3 significant figures. Time will also be presented to 3 significant figures.

- For safety summaries, means and medians will be displayed to one more decimal place than the original data, SD will be displayed to two more decimal places, and the minimum and maximum will be displayed to the same number of decimal places. Percentages will be reported with exactly one decimal place, with the exception of 100% which will be reported without a decimal place.

- Derived data where it is known in advance the result will be an integer for example number of days will be presented with zero decimal places.

6.7 Conventions for Missing and Partial Dates

It is not expected that there will be any missing dates, however in the rare case that an Adverse Event (AE) start date or time is missing and it is unclear whether the AE is treatment emergent or not then a conservative approach will be taken and it will be assumed that the AE occurred after first dosing.

All dates presented in the individual subject listings will be as recorded on the eCRF.

6.8 Handling of Missing Data

Missing data are handled as follows:

- Missing values for individual data points will remain as missing. Missing values will not be imputed for data and only observed values will be used in safety and efficacy data analyses and presentations. If there is missing data for PK assessments from the central lab at a specific timepoint, the local lab result for that timepoint will be used for analysis.

- Where individual data points are missing, categorical data will be summarized based on reduced denominators (that is, only patients with available data will be included in the denominators).

- Handling of below the limit of quantitation (BLQ) plasma concentration values is defined under Section 7.9 Pharmacokinetic Assessments.

7.0 STATISTICAL ANALYSES

7.1 Patient Disposition

Patient disposition information will be summarized for all patients as follows:

- The number of patients completing the study drug, the reason for discontinuation of study drug, the number of patients completing the study, and the reason for discontinuation of study will be summarized by dose cohort and overall.

- The number of randomized patients and number of patients in each analysis population by dose level, cohort and overall.

A listing of all patients who prematurely discontinued from study drug or prematurely withdrew from the study will be presented, and the primary reason for discontinuation of study drug or withdrawal from the study will be provided. And a listing of patients excluded from the analysis populations and the reason for their exclusion will be provided.

7.2 Protocol Deviations

In accordance with ICH E3, Sponsor-defined eligibility violations and important post-randomization protocol deviations will be identified and listed separately by patient.

Deviation type/code as provided by Sponsor:

- Eligibility criteria related
- Study procedure not done
- Study procedure not per protocol
- Withdrawal criteria
- Missed study visit
- Visit out of study window
- Patient not withdrawn as per protocol
- Prohibited medication/therapy
- Informed consent
- Laboratory samples
- Safety reporting

- Study drug administration
- Study drug storage and handling
- Other

A listing of protocol deviations will be provided.

7.3 Demographics and Baseline Characteristics

Demographic data and baseline characteristics will be presented for the Safety, Efficacy and PK populations. A table will present the patient demographics (that is, gender, age, ethnicity and race) and baseline characteristics of height (cm), weight (kg) and BMI (kg/m^2), collected before the start of study drug. Additionally, the baseline Phe values will be summarized under baseline characteristics.

A demographic listing, which includes the date the informed consent was signed, will be provided.

7.4 Medical History

Medical history collected at screening will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) classification, version 23.1. Medical history will be summarized by system organ class (SOC) and preferred term (PT). Patients who report 2 or more medical history items that are coded to the same SOC and/or PT will be counted only once by the unique coded term in the summary. The summary will be provided for the Safety population.

A listing will be provided by patient for the Safety population.

7.5 Prior and Concomitant Medications

Verbatim terms on eCRFs will be mapped to Anatomical Therapeutic Chemical (ATC) class and Generic Drug Names using the World Health Organization (WHO) (WHODrug Enhanced September 2020) dictionary.

Prior medications are those medications that discontinued before the start of study drug. Concomitant medications are those medications taken at the start of study drug or initiated after the initial dose of study drug, or medications that were initiated prior to the start of study drug and continue to be taken after study drug is administered.

Prior medications and concomitant medications will be summarized by ATC level 1 term, ATC level 3 term and Preferred Term (generic name) with frequency and percentage of patients in each dosing arm using each prior medication and each concomitant medication. At each level of patient summarization, a patient is counted once if he/she reported one or more medications at

that level. Each summary will be ordered by descending patient count in the overall column by each level of ATC class and generic drug name within each level of ATC class. The summary of prior and concomitant medications will be provided for the Safety population.

Prior and concomitant medications will also be listed separately. Those medications that are initiated after the last dose of study drug will be flagged in the concomitant medication listing.

7.6 Extent of Exposure

Extent of exposure to study drug will be examined by assessing days dosed.

Days dosed will be defined as the number of days during which the patient received any amount of study drug to be administered orally (powder of suspension) and will be summarized for days dosed by period and then overall.

7.7 Efficacy Analyses

All efficacy analyses will be performed on the Efficacy population.

7.7.1 Efficacy Outcomes

- Change in Phe levels with respect to baseline

  Phe concentrations at each visit and changes from baseline (Day 1, pre-dose) to each post-Day 1 assessment will be summarized using summary statistics within each dose cohort and period. The primary efficacy outcome is the reduction in plasma Phe levels defined as the change of plasma Phe concentrations at Day 7 from baseline (Day 1, pre-dose) within the same treatment period. 95% confidence intervals will be calculated for the change in baseline to Day 7 Phe values for each period within a cohort.

- Number and percent of patients with Phe concentrations in acceptable treatment range of 130 to 360 µmol/L at Day 7
- Number and percent of patients with Phe concentrations <130 µmol/L at Day 7

7.7.2 Exploratory Outcomes

Exploratory biomarker, prolactin and serotonin outcomes will be summarized by visit along with changes from baseline within each cohort and period. A listing will be provided by patient for the Safety population.

- Urine sepiapterin, neopterin, and BH_4
- Serum prolactin

- Whole blood serotonin

Cognitive impairment as measured using the CANTAB will be summarized at Screening, Day 1 (pre-dose) and Day 7 of each treatment period. The CANTAB test components of RTI, SSP, SWM and RVP will be represented graphically through time by cohort to assess chronological and treatment effects if any. Each component of the CANTAB will be summarized at each visit and also changes from baseline, if possible. A listing will be provided by patient for the Safety population.

Changes in physical activity and sleep patterns of behavior will be recorded using wrist-worn GENEActiv accelerometer. A listing will be provided by patient for the Safety population.

NIH Toolbox Cognition Domain will be used to evaluate the changes in cognitive assessment from baseline. The Toolbox was specifically designed to measure neuropsychological and behavioral functions over time and to measure key constructs across developmental stages. This facilitates the potential evaluation of treatment effectiveness of CNSA-001, as well. This study will use an age-based battery of assessments from the Cognitive Domain of the NIH Toolbox. All tests will be administered via an iPad on either Day-1 or Day 1 predose (that is, at baseline) and on Day 7 of each treatment period. A listing will be provided by patient for the Safety population.

Child and Adolescent Sleep Checklist: A questionnaire will be provided to identify sleep problems in children, ages 3-18 years. Parents or caregivers are asked to recall the child's sleep during the last 7 days when providing responses to questions. Responses to the questions about sleep habits are scored from 0 (never or don't know) to 3 (always), yielding an overall score of 0-72. Scores above 18 indicate sleep problems. In addition, there are four categories of scores: Bedtime problems (Q1-Q6; 6 questions), sleep breathing and unstable sleep (Q7-Q12; 6 questions), parasomnia and sleep movement (Q13-Q18; 6 questions), and daytime problems (Q19-Q24; 6 questions). A listing will be provided by patient for the Safety population.

7.8 Safety Analyses

All safety analyses will be performed on the Safety population.

7.8.1 Adverse Events (AEs)

Adverse events will be coded to SOC and PT using MedDRA version 23.1. The coding process will be described in the Data Management Plan.

Summaries will be provided for AEs, with the number and percentage of patients reporting each type of event presented. If a patient reports the same PT more than once, it is counted only once within that category. A similar comment applies to the SOC; namely if a patient has AEs of two or more PTs under the same SOC, then that patient only counts once for that SOC. Further, for a

given summary, the PT will only be counted once at its worst severity and strongest relationship to treatment.

Pre-treatment AEs are defined as AEs that have an onset prior to the time of the first dose and after the informed consent date.

AEs will be regarded as TEAEs if they start on or after first dose of study drug administration and no more than 7 days after the last dose of study drug (30 days after last dose of study drug for SAEs). The following rules will be used to assign a TEAE to a treatment group:

- A TEAE will be assigned to the treatment received immediately before onset.
- Any TEAE reported within the washout period between doses will be attributed to the previous treatment.
- If the severity of a TEAE increases in a later period, the TEAE at the increased severity will be assigned to the treatment received immediately before the increase in severity.

The following summaries for TEAEs will be provided:

  - An overall summary table of TEAEs summarizing the number and percent of patients, in the following categories: any TEAE, severe TEAE, TEAE related to study drug, serious TEAE, TEAE's leading to premature discontinuation of study drug and TEAEs leading to discontinuation of study.
  - Incidence of TEAEs by MedDRA SOC and PT.
  - Incidence of TEAEs by MedDRA SOC and PT, and worst severity.
  - Incidence of TEAEs by MedDRA SOC and PT, and strongest relationship to study drug.
  - Listing of All SAEs
  - Listing of All AEs for patients who prematurely discontinued study drug or prematurely withdrew from the study due to an AE.
  - Listing of All AEs for patients who died.
  - Incidence of serious TEAEs (SAEs) by MedDRA SOC and PT.

In addition to the by-treatment summaries described above, a data listing will be provided for all AEs (including a TEAE flag).

7.8.2 Clinical Laboratory Evaluations

Descriptive statistics for chemistry, hematology and urinalysis parameter values and the change from baseline at will be summarized for the Safety population. Change from baseline will be calculated for each patient at each post-baseline visit.

Additionally, shift in liver parameters from baseline to the highest post-baseline result (worsening) will be presented for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), Bilirubin and gamma glutamyl transferase (GGT); these will be based on the provided laboratory reference ranges for high and low.

A listing of hematology, chemistry and urinalysis laboratory measurements will be provided.

7.8.3 ECG Parameters

Descriptive statistics for ECG parameters (that is, Heart Rate [HR], RR interval, PR interval, QRS duration, QT interval, corrected QT interval [QTc] and QTc calculated using Fridericia's formula [QTcF]) at baseline and the Day 7 visit and the change from baseline will be presented. Given that triplicate readings of ECGs will be collected, where each reading is taken 1 minute apart, means will be calculated on these multiple ECG measurements (by time point), and the table summaries will be based on these means.

For QTcF, a distribution of the increase from baseline to Day 7 on the categories <0 msec, ≥1 to <30 msec, ≥30 to <60 msec, ≥60 to <90 msec, and >=90 msec will be presented; as well as a distribution on the categories ≤ 450 msec, > 450 to ≤ 480 msec, >480 to ≤ 500 msec, and >500 msec. If needed, QTcFs will be calculated using the following formula: $QT/(RR)^{1/3}$.

Listings of electrocardiogram data will be provided, including the normal, abnormal and clinically significant status of each measurements.

7.8.4 Vital Signs

Systolic and diastolic blood pressures (SBP and DBP), HR, respiratory rate (RR) and body temperature, will be summarized using descriptive statistics at each time point at which they were measured. Temperature will be collected in °C (Celsius) or °F (Fahrenheit) in eCRF, all values will be converted to °C for analysis using the formula °C = (°F – 32) * 5/9.

Descriptive statistics of the change from baseline to each post-baseline time point will also be provided.

A summary of abnormal values based on absolute and relative criteria, identified by the criteria provided in the table below, will be provided for patients with both baseline and post-baseline values. The summary table will have two sets of summary statistics for each parameter; one row of summaries for the absolute criterion and then another row based on the more restrictive summaries of absolute and relative criterion (both must be met). In the table that follows absolute criteria are provided in the 3rd column and relative criterion is contained in the last column.

All vital sign parameters will also be provided in listings.

Criteria for Treatment Emergent Clinically Significant Vital Signs

Vital Sign Parameter	Flag (*)	Criterion Value	Change from Baseline
Systolic Blood Pressure (mmHg)	High (CH)	≥ 180	Increase of ≥ 20 mmHg
	Low (CL)	≤ 90	Decrease of ≥ 20 mmHg
Diastolic Blood Pressure (mmHg)	High (CH)	≥ 105	Increase of ≥ 15 mmHg
	Low (CL)	≤ 50	Decrease of ≥ 15 mmHg
Heart Rate (bpm)	High (CH)	≥ 120	Increase of ≥ 15 bpm
	Low (CL)	≤ 50	Decrease of ≥ 15 bpm

(*) CH = clinically high; CL = clinically low.

7.8.5 Physical Examinations

The percentage of patients with an abnormal body system examination at baseline and each post-baseline visit will be summarized by body system for patients on the Safety population. Physical examination results will also be presented in listings.

7.9 Pharmacokinetic Assessments

Sampling for PK analysis (with evaluation of sepiapterin and its effect on BH_4, Phe and Tyr levels) will occur at the following time points for each dose level: on Day 1 at pre-dose (within 30 min of dosing), +0.5 hr (± 3 min), +1 hr (± 5 min), +2 hr (±6 min), +4 hr (± 20 min), +6 hr (± 30 min), +8 hr (±60 min, prior to Day 1 evening dose), and +24 hours (±2 hr, prior to Day 2 morning dose) after the first dose of study drug.

For these analyses, the patients will be analyzed using the PK population (as per the treatment received). The concentration of CNSA-001 (sepiapterin), BH_4, Phe, and Tyr in plasma samples will be analyzed by MNG Laboratories using a validated LC/MS/MS method. The following non-compartmental PK parameters will be estimated and listed along with graphical presentation

of the concentration data (note: additional PK parameters may be calculated as needed to adequately characterize the PK of sepiapterin and associated analytes):

Parameter	Definition
$AUC_{0\text{-last}}$	Area under the curve from time 0 to the time of the last quantifiable concentration trapezoidal method (linear-up/log-down method)
Tlast	Time of last observed quantifiable concentration
C_{max}	Maximum observed concentration
T_{max}	Time to C_{max}

Data Considerations: Plasma concentration values that are below the lower limit of quantitation (BLQ) will be presented as "BLQ" in the concentration data listing. For noncompartmental analysis BLQ values are handled as follows:

- BLQ values prior to the first quantifiable concentration will be treated as 0.
- BLQ values appearing between two quantifiable concentrations will be treated as missing.
- BLQ values in the terminal elimination phase are treated as missing. Once two BLQ values are observed in the terminal phase, the profile is deemed to have terminated and all subsequent quantifiable values are treated as missing.

PK Graphs:
For each of the following analytes: sepiapterin, BH_4, Phe, and Tyr, figures will be derived and will include, individual patient PK profiles, mean PK profiles and as needed box plots or scatter plots comparing derived PK.

PK concentrations time graphs:
For sepiapterin, BH_4, Phe, and Tyr analytes, the following linear and semi-log graphs will be presented:

- Individual (patient) PK profiles by dose level
- "Spaghetti plots" displaying all individual profiles together, and
- Mean/Median plots comparing PK profiles across all dose levels

Nominal blood sampling times will be used to calculate the median and mean concentrations at each time point.

PK Listings: Detailed patient listings of plasma PK results will be provided.

Appendix A: Schedule of Assessments and Procedures

Evaluation	Screen Period	BH4 Washout	Treatment Period									End of Study (≤ 48 hrs after last dose)	Phone Follow-up	
			Period 1				Washout	Period 2						
	Day-14 to Day -4	Day -3 to Day -1	Day 1	Day 2	Day 4 (±1)	Day 7	3 (±1) days	Day 1	Day 2	Day 4 (±1)	Day 7		7 - 10 days after last dose	30 (±3) days after last dose
Informed consent	X													
Confirm inclusion/exclusion criteria eligibility	X		X[a]					X[a]						
Confirm mutation analysis for PBD genes [b]	X													
Demographics	X													
Medical history [c]	X													
Vital signs, height and weight [d]	X		X[a]	X		X		X[a]	X		X	X		
ECGs [e]	X		X[a]			X		X[a]			X			
Physical examination [f]	X		X[a]			X		X[a]			X	X[s]		
Clinical laboratory tests and, serum prolactin [h]	X		X[a]			X		X[a]			X	X[s]		
Serum/urine pregnancy test [i]	X		X[a]					X[a]				X		
Prior/Concomitant meds [j]	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse events [k]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Phe concentrations [l]	X	X	X[a]	X[a]	X	X		X[a]	X[a]	X	X	X		
Consistent diet/ 3-day diet record [m]		X	X	→		X	X	X	→		X	X[s]		
Seizure journal	X	X	X	X	X	X	X	X	X	X	X	X		
Discontinue BH4 [n]		X	→											
PK: Sepiapterin, BH4, and Phe/Tyr [o]			X[a]	X[a]	X	X		X[a]	X[a]	X	X	X		
Biomarkers in urine [p]	X		X[a]	X[a]	X	X		X[a]	X[a]	X	X			
Whole blood serotonin	X		X[a]	X[a]	X	X		X[a]	X[a]	X	X			
CANTAB testing [q]	X	X	X[a]			X		X[a]			X			
Don GENEActive Accelerometer [u]	X	X	X	X	X	X	X	X	X	X	X	X		
NIH ToolBox Test Battery		X[a,v]	X[a,v]			X		X			X			
Administer the CASC			X			X		X			X			
Administer study drug [r]			→					→						

Dispense study drug [s]				X					X					
Dispense Dosing Diary [t]				X					X					
Collect Study Drug, Dosing Diary, Assess Compliance						X					X	X		
Resume BH4 therapy												X	X	X

a. To be completed prior to initial dosing.

b. Confirmed diagnosis of PBD as evidenced by medical history of biallelic pathogenic mutations in PTPS or recessive GTP-CH genes, abnormal enzyme activity of the PTPS or GTP-CH enzymes, or a CSF biochemical profile indicative of PTPS or GTP-CH deficiencies. No genetic testing or retesting is required.

c. Includes specific information related to any prior or existing medical conditions or surgical procedures involving the following systems: dermatologic; HEENT; lymphatic; cardiovascular; respiratory; gastrointestinal; musculoskeletal; and neurological. Also, includes documentation of 3 most recent historical Phe concentrations.

d. Includes blood pressure, pulse, respiratory rate, and oral temperature. Patients will have a 5-minute rest in a supine position before vital signs are assessed. Weight will only be collected at Screening and on Day 1 of each treatment period. Height will only be collected at Screening. Vital signs will be collected both pre-dose and 2-hours post-dose (before 2-hour blood collection for PK) on Day 1 of each treatment period; for all other timepoints they will be taken at any time during the visit.

e. 12-lead ECGs to be performed at Screening, and on Day 1 (pre-dose) and Day 7 of each treatment period. ECGs will be performed in triplicate, with each read taken 1 minute apart.

f. The examination will assess general appearance, as well as dermatologic, HEENT, lymphatic, cardiovascular, respiratory, gastrointestinal, musculoskeletal, and neurological parameters.

g. Can be performed either on Day 7 visit of Treatment Period 2 or at the EOS Visit. Does not need to be conducted at both.

h. Includes clinical chemistry panel (albumin, AP, ALT, AST, BUN, calcium, CO2, chloride, creatinine, GGT, glucose, LDH, phosphorus, potassium, sodium, total bilirubin, direct bilirubin, total cholesterol, total protein, uric acid), and serum prolactin; hematology panel (hematocrit, hemoglobin, platelet count, RBC, WBC, and WBC differential); and urinalysis (appearance, bilirubin, color, glucose, ketones, microscopic examination of sediment, occult blood, pH, protein, specific gravity, and urobilinogen).

i. Serum and urine pregnancy test required for all women who are not postmenopausal and for adolescents who have started menstruation; serum testing to occur during the Screening Period and urine testing to occur prior to dosing on Day 1 of Periods 1 and 2 and EOS. A positive urine test must be confirmed with a serum test.

j. Record all Rx and OTC medications starting from 30 days prior to Screening through last study visit.

k. Adverse events are to be collected from the time of informed consent until 7 days after the last dose of study drug (30 days after last dose for SAEs).

l. Blood Phe concentrations collected during Screening (prior to BH4 Washout) and during BH4 Washout Period on Days -3 and -1 will be analyzed by the site's local laboratory. To minimize burden on patients, Phe collection on Days -3 and -1 during the Washout Period may be obtained via filter paper with overnight shipping to the site's designated laboratory. All other blood Phe concentrations will be used for safety monitoring and analyzed by the site's local laboratory and are collected on Day 1 and Day 2 (pre-dose), and on Day 4 (±1 day) and Day 7 (after the AM dose) during Period 1 and Period 2 of Study Treatment, and EOS visit. Unscheduled Phe collection may occur if at any time a patient is hypophenylalaninemic (i.e., <30 µmol/L). Phe collection should be performed with pre-fed or at least 3 hours following last meal and at approximately the same time of day at each visit.

m. Patients are to maintain a consistent diet (with respect to protein and Phe intake) during the study. The 3-day diet records will be collected during BH4 Washout and during each treatment period.

n. BH4 treatment [i.e., Kuvan® (sapropterin dihydrochloride)] will be discontinued starting with the BH4 Washout period. Patients will remain off BH4 treatment until the EOS visit.

o. Blood samples for CNSA-001 (sepiapterin), BH4, Phe, and Tyr will be collected and analyzed by the bioanalytical laboratory at the following timepoints during Period 1 and Period 2: Day 1 (pre-dose, within 30 min of dosing), +0.5 hr (±3 min), +1 hr (±5 min), +2 hr (±6 min), +4 hr (±20 min), +6 hr (±30 min), and +8 hr (±60 min, prior to Day 1 evening dose), and +24 hours (± 2 hr, prior to Day 2 AM dose) after the first dose of study drug; +72 hr (Day 4, ±1 day; Phe/Tyr only); on Day 7 (Phe/Tyr only); and EOS (Phe/Tyr only) (see Table 2). Phe concentrations for preliminary efficacy will be obtained from the same sample collected for Phe/Tyr concentrations on Day 1 and Day 2 (pre-dose), Day 4 (±1 day) and Day 7 (after the AM dose), and EOS.

p. Exploratory biomarkers in urine include sepiapterin, BH4, and neopterin. These samples will be collected at Screening, pre-dose on Day 1 and Day 2, and post-dose on Day 4 (±1 day) and Day 7. Urine samples should be the first void of the day. Samples will be sent to the bioanalytical laboratory (see Table 3).

q. CANTAB testing will include RTI, SSP, SWM, and RVP tests. These will be administered at the Screening visit and on Day 1 (pre-dose) and Day 7 of each treatment period.

r. Study drug will be administered twice daily (with breakfast and with supper) on Days 1 to 7 of each treatment period. Doses on Day 1 and Day 2 (AM only) will be administered by research staff, while all remaining doses will be self-administered.

s. Study drug will be dispensed at each Day 2 Study visit and will include enough study drug for dosing until the Day 7 study visit. CNSA-001 treatment will be discontinued if criteria related to safety or lack of efficacy are met (see Section 6.13.1).

t. Patient will receive a dosing diary and instructions for recording all doses of study drug taken and times they were taken; if a patient vomits after taking a dose of study drug, this should also be recorded in the diary and the patient should wait until the next scheduled time point to administer a dose.

u. Patients will be asked to wear a GENEActiv accelerometer device continuously for up to two 14-day periods. The initial device will be placed on the patient's wrist, ankle or other suitable location and secured with the appropriate band no earlier than Day -7 during Screening. The initial device will be removed on Day 7 of Treatment Period 1 and replaced with a second device to be worn until the End of Study visit. All devices will be collected at the End of Study visit.

v. Patients ages 3-17 will be administered the NIH Toolbox Cognitive Domain test battery on either Day -1 or Day 1 predose for Treatment Period 1 at the clinical site's option. Only one assessment will be performed and will be used as the baseline assessment for Treatment Period 1.

Abbreviations: AE = adverse event; AM = morning; AP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase; BUN = blood urea nitrogen; CO2 = carbon dioxide; HEENT=hear, eyes, ears, nose, throat; ECG = electrocardiogram; EOS = end of study; GGT = gamma glutamyl transferase; LDH = lactate dehydrogenase; Phe = phenylalanine; PK = pharmacokinetic; RBC = red blood cell; RVP = Rapid Visual Information Processing; SAE = serious adverse event; RTI = Reaction Time; SSP = Spatial Span; SWM = Spatial Working Memory; WBC = white blood cell.

Appendix B: Group Labels Used in Table, Figure and Listing Deliverables.

	Cohort 1		Cohort 2	
Group labels used for TFLs:	**CNSA-001 2.5 mg/kg**	**CNSA-001 10 mg/kg**	**CNSA-001 5 mg/kg**	**CNSA-001 20 mg/kg**
Matching Protocol group definitions:	2.5 mg/kg/day for 7 days for Cohort 1 in Period 1	escalate to 10 mg/kg/day for 7 days for Cohort 1 in Period 2	5 mg/kg/day for 7 days for Cohort 2 in Period 1	escalate to 20 mg/kg/day for 7 days for Cohort 2 in Period 2

The chemical name and structure of sepiapterin, or PTC923 (formerly known as CNSA-001), has been presented in various ways in the published literature. One such chemical name and structure was included in the Protocols included in PBD-001 CSR Section 16.1.1. However, the preferred representation of the active agent is as follows:

Chemical name: (S)-2-amino-6-(2-hydroxypropanoyl)-7,8-dihydropteridin-4(3H)-one

Chemical structure: