Official Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

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Applicant/MAH: BioMarin Pharmaceutical Inc.

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105 Digital Drive

Novato, CA 94949

Statistical Analysis Plan, Version 0

Study 165-306

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1 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
C3	complement component 3
C4	complement component 4
CRP	C-reactive protein
CRF	Case Report Form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	diastolic blood pressure
ECG	electrocardiogram
HAE	hypersensitivity adverse event
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
ITT	intent-to-treat
IWRS	Interactive Web Response System
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
Nab	neutralizing antibody
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
PAL	phenylalanine
PEG	polyethylene glycol
Phe	phenylalanine
PK	pharmacokinetics
PKU	phenylketonuria
PT	preferred term

SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
SOC	system organ class
SMQ	Standardized MedDRA Queries
TEAE	treatment-emergent adverse event
Tab	total antibody
WHO Drug	World Health Organization Drug Dictionary

2 INTRODUCTION

Study 306 is an open-label, two-arm (Active vs Diet-only Control), randomized control study to evaluate safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent subjects with phenylketonuria (PKU).

The purpose of this Statistical Analysis Plan (SAP) is to provide a comprehensive description of methods of the data analyses outlined in the protocol (22 September 2021).

If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the SAP will prevail.

2.1 Objectives of Study

The primary objective of the study is:

- To evaluate the efficacy of pegvaliase in adolescent subjects with PKU
- To evaluate the safety of pegvaliase in adolescent subjects with PKU

The secondary objectives of the study are:

- To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment
- To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU

The exploratory objectives of the study are:

- To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU
- Explore the biochemical, molecular, and cellular aspects of PKU
- PAH genotyping

2.2 Study Design

This is an open-label, two arm, randomized control study. Approximately 54 subjects 12-17 years old, inclusive, with diet-only control will be enrolled. At enrollment, subjects will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 subjects receiving daily pegvaliase and 18 subjects managing PKU by diet alone. Treatment assignment will be stratified by blood Phe ≤ 1000 µmol/L or > 1000 µmol/L based on an average of the 2 Screening blood Phe assessments. Subjects in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), subjects will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for subjects in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of an Induction/Titration Phase (9 weeks) and a Maintenance Phase. Required clinic visits during Part 1 will occur weekly for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 40 mg/day or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active subjects only), and safety assessments.

Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 80 additional weeks (through Week 153). Beginning Week 73 the subjects in the control treatment arm will initiate pegvaliase treatment and follow a parallel schedule for dosing, study visits, and assessments in Weeks 73 through 145 to that followed by the active arm subjects during the Primary Treatment Phase (Part 1) from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

2.3 Study Population

Individuals with PKU aged 12 to 17 years old, inclusive, and diet-only control at the start of the Screening/Run-in Period (Day -28) are candidates for participation in the study. Additional criteria for study participation are presented in Protocol 165-306 Section 5.1 and Section 5.2.

2.4 Study Dosage and Administration

Subjects will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

2.5 Sample Size Determination

Approximately 54 subjects will be enrolled into the study and randomized to the pegvaliase or diet-only treatment in 2:1 ratio. Assuming a mean (SD) reduction in Phe at Week 73 of

640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided).

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

For the pegvaliase treatment arm, the mean (SD) assumption of reduction in blood Phe is based on subjects 16 to 24 years of age who completed 17 months of treatment in previous pegvaliase studies utilizing an I/T/M dosing regimen (n = 46). For the diet-only arm, the mean (SD) assumption of reduction in blood Phe is based on BioMarin's PKUDOS (Phenylketonuria Demographic, Outcomes, and Safety) registry for patients aged 12 to <18 years old on diet only control with baseline Phe > 600 µmol/L at year 1 of study enrollment (n = 57).

2.6 Blinding and Randomization Methods

2.6.1 Blinding Method

Subjects will receive open-label pegvaliase or diet-only treatment.

2.6.2 Randomization Method

Subjects will be randomized in a 2:1 ratio to the active (pegvaliase) or control (diet-only) treatment arm, respectively. Treatment assignment will be stratified by blood Phe ≤ 1000 µmol/L or > 1000 µmol/L based on an average of the 2 Screening blood Phe assessments.

2.7 Interim Analysis

There are no interim analyses planned. The primary analysis will be performed after subjects completed Part 1 or discontinued from the study. The final analysis will be performed after subjects have completed the study or discontinued from the study.

3 GENERAL ANALYSIS CONSIDERATION

Descriptive summaries of continuous variables will include the mean, standard deviation (SD), median, minimum, and maximum. Descriptive summaries of categorical variables will include n, frequency, and percentage.

3.1 Analysis Populations

The All Enrolled population will consist of all subjects (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.

The Intent to Treat (ITT) population will consist of all subjects who are randomized. Subjects will be analyzed according to the randomized treatment.

The Safety population will consist of all subjects who received study treatment (pegvaliase or diet-only management of PKU). Subjects will be analyzed according to the treatment actually received during Part 1.

The Per Protocol (PP) population will consist of all subjects in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. The PP population will be determined by the study team through data review before database lock for primary analysis; reasons for excluding subjects will be defined and documented prior to database lock for primary analysis.

The PK population will consist of all subjects with at least one PK measurement.

3.2 Treatment Group Presentation

The following treatment groups will be used in summary tables:

Part 1: pegvaliase (active) or diet-only (control)

Part 2: pegvaliase (subjects continuing on pegvaliase) or diet-only (subjects transitioning from control to pegvaliase)

3.3 Pooling of Data from Sites with Small Enrollment

Since the enrollment for each site is expected to be small, the analyses will not be adjusted by site or pooled sites.

3.4 Study Day Derivation

Study days will be derived as follows:

Pegvaliase arm: the first dose date will be assigned as Study Day 1

Diet only arm: the Visit 1 date will be assigned as Study Day 1

For visit occurs on or after Study Day 1, Study Day = visit date – day 1 date + 1

For visit occurs before Study Day 1, Study Day = visit date – day 1 date. There is no Study Day 0.

3.4.1 Baseline Definition

The baseline for all safety and efficacy endpoints (when applicable) is defined as the last non-missing measurement collected prior to the first pegvaliase dose (pegvaliase arm) or prior to or on Visit 1 date (diet-only arm), unless otherwise specified.

Baseline blood Phe concentration is defined as the average of 3 pre-treatment measurements collected between screening/Run-in and Day 1 pre-dose.

Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in.

3.5 Visit Window for Analysis

All efficacy and safety data will be summarized by study week of assessment. An assessment for a subject will be classified according to the study day of the assessment where it falls within a window. The windows are designated for each scheduled week of visit and centered on a target day. If there are two or more assessments within a designated window, the assessment that is closest to the target day will be used for analyses. If the two closest assessments to the target day are equidistant from the target day, then for numerical variable, the average of the two assessments will be used for analyses. For categorical variables, the worst of the 2 equidistant measurements, indicating less treatment effect, will be used.

Table 3.5.1~~Table 3.5.1~~ lists the scheduled weeks of the assessments collected in this study and the corresponding range of treatment days (window) during which a visit may have occurred.

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Table 3.5.1: Visit Time Windows for the Treatment Period

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (XX ≥ 13, every four weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 - 2 to Day (XX – 1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤ XX ≤ 69, every four weeks)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 -13 to Day (XX -1)*7 + 14
	Week 73	Day 505	Day 491 to Day 508
Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3
	Week 81	Week 73 day + (81 – 73)*7	Week 73 day + (81 - 73)*7 - 3 to Week 73 day + (81 - 73)*7 + 13
	Week XX (XX > 81, every four weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 14 to Week 73 day + (XX - 73)*7 + 13

[a] Target days are relative to the Study Day 1.

[b] Visit day is calculated by (visit date – Study Day 1 + 1).

Diet only group: all Part 2 visit is relative to the first dose day of pegvaliase

3.6 Handling of Dropouts and Missing Data

Subjects who discontinued prematurely will not be replaced.

Missing dates or partially missing dates will be imputed conservatively for concomitant medications and AEs to ensure that an AE is considered treatment emergent and has the longest possible duration. Missing data in blood Phe will be handled by multiple imputation method, more details are provided in Section 12.1.1

4 SUBJECT DISPOSITION

The total number of enrolled subjects, the number (%) of subjects who received study treatment, the number (%) of subjects completed Part 1, the number (%) of subjects who completed the study, and the number of subjects in each analysis population (ITT, Safety, PP and PK), as appropriate, will be summarized.

5 DISCONTINUATION AND COMPLETION

For subjects who prematurely discontinued the study, the primary reason for discontinuation will be summarized by treatment group. A similar summary will be provided for subjects who discontinued study treatment. A subject listing of completion and early termination from study will also be provided.

6 PROTOCOL DEVIATIONS

Both major and minor protocol deviations will also be summarized by deviation category. All protocol deviations will be listed. Subjects with inclusion or exclusion criteria deviations will also be provided in a separate listing.

7 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Subject demographic and baseline characteristics will be summarized for the ITT population. Demographics include age, age category (12 to 15 / 16 to 17 years old), gender, race, country and ethnicity. Baseline characteristics include height, weight, BMI, blood Phe concentration, neurocognitive assessment, three-day average dietary intake, restricted diet, genotyping and antibody status.

8 MEDICAL HISTORY

Each subject's chronic diseases, disorders, and surgeries in the past will be collected as general medical history. General medical history will be summarized by System Organ Class (SOC) and Preferred Term (PT) by descending order of frequency. A by-subject listing of each subject's medical history will also be provided.

9 PRIOR AND CONCOMITANT MEDICATIONS/PROCEDURES

The following definitions will be used to determine prior and concomitant medications:

- Prior medications: any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Concomitant medications: any medications initially taken on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Prior and concomitant medications: any medications taken both prior to and on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) will be reported both as prior and concomitant medications. Therefore, any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) where the stop date is reported as "continuing" or missing will be considered as both prior and concomitant medications.

In the event the start date of a medication is partial, the following imputation rules will be applied:

If only day is missing, then the start date will be imputed as the first day of the month, if the year and month is same or after the first dose year and month. If only day is missing, then the start date will be imputed as the last day of the month, if the year and month is prior to the first dose year and month. If month is the same as the month of study day 1 then the start date will be imputed as the date of study day 1.

- If only year is non-missing, then the start date will be imputed as the first day of the year. If year is the same as the year of study day 1 then the start date will be imputed as the date of study day 1.

In the event the stop date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the end date will be imputed as the last day of the month.
- If only year is non-missing, then the end date will be imputed as the last day of the year.

All medications will be coded using the current version of the World Health Organization Drug (WHO Drug) dictionary (MMM YYYY). Prior and concomitant medication use will be separately summarized by Anatomical Therapeutic Chemical (ATC) medication class (Level 4) and preferred name (ie, generic medication name). A subject reporting the same medication use more than once will be counted once when calculating the number and percentage of subjects who received that medication. A subject listing of each subject's prior and concomitant medications will be provided.

10 STUDY DRUG USAGE

Study drug usage rate will be calculated based on the drug exposure records captured in eCRFs and dispensed drug amount captured in the Interactive web response system (IWRS).

The overall study drug usage rate will be derived from the total amount of study drug intake divided by the total dispensed study drug amount over the study period and multiplied by 100%. The rate will be calculated for Part 1, Part 2 and overall study period.

11 EXTENT OF EXPOSURE TO STUDY TREATMENT

For pegvaliase treatment period, the total number of days on study treatment, total amount of study drug (mg) taken and average daily dose (mg) will be summarized for Part 1, Part 2 and overall study period. Any dose interruption longer than 28 days will be excluded from the total exposure duration calculation.

In addition, the total number of days on diet control treatment will be summarized for pegvaliase and diet-only arm, the summary of protein intake is described in Section 12.2.1

A by-subject listing of each subject's extent of exposure and compliance will be provided.

12 EFFICACY EVALUATIONS

Unless otherwise specified, efficacy analysis will be based on the ITT population.

12.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study, which is defined as the average of Week 69 and Week 73 blood Phe measurements – baseline blood Phe. Change from baseline in blood Phe will be compared between subjects in the pegvaliase arm and the diet-only arm.

12.1.1 Primary Estimand

Population: the target study population comprises adolescent subjects (ages 12-17) with PKU who meet the inclusion and exclusion criteria as specified in the protocol. The analysis population is the ITT population as defined in Section 3.1

Variable: the variable is the primary efficacy endpoint, change from baseline in blood Phe following 72 weeks on study, as defined in Section 12.1.

Handling of missing data and intercurrent events (ICEs): the treatment policy will be adopted which will include all blood Phe data regardless of intercurrent events such as treatment discontinuation. Missing blood Phe data will be imputed by multiple imputation methods as described in Section 12.1.2.

Population level summary: the difference in change from baseline in blood Phe following 72 weeks on study will be estimated by an Analysis of Covariance (ANCOVA) model with multiple imputation of missing data, as described in Section 12.1.2.

12.1.2 Primary Analysis

The primary efficacy endpoint will be evaluated using an ANCOVA model, which will include baseline blood Phe as covariates and treatment group as factor, with missing data handled by multiple imputation. The imputation will be performed using the SAS procedure PROC MI; the variable list for imputation will include all baseline and post-baseline blood Phe measurements and stratified by treatment. The MCMC method will be used to generate 100 imputation datasets. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. The primary analysis will be based on the ITT population.

Descriptive summary of observed values, change and percent change from baseline in blood Phe will be provided by visit and treatment. In addition, categorical summary will be performed for the following categories:

Percent of subjects with blood Phe <= 120 µmol/L at each visit during study

Percent of subjects with blood Phe <= 360 µmol/L at each visit during study

Percent of subjects with blood Phe <= 600 µmol/L at each visit during study

In Part 2, change and percent change in blood Phe from average of Week 69 and Week 73 visits will also be summarized for subjects transitioning from control to pegvaliase.

12.1.3 Sensitivity Analysis

The same statistical model used in the primary analysis will be repeated in the PP population.

12.2 Secondary Endpoints

The secondary endpoints are as follows:

Change from baseline in total dietary protein intake (i.e., medical food and/or intact food). The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits.

Plasma sample for trough PK

12.2.1 Analysis on Secondary Endpoints

For the change from baseline in total dietary protein intake, the observed values, change and percent change from baseline at each visit will be summarized. Similar summary will also be provided for protein intake from medical food and from intact food separately. The values collected on the three-day diet diary will be averaged for each parameter at each visit before being summarized.

In Part 2, change and percent change in dietary protein intake from average of Week 69 and Week 73 visits will also be summarized for subjects transitioning from control to pegvaliase.

For plasma sample of trough PK, table summary and subject listing of observed value will be provided at each visit. Details of PK analysis of pegvaliase concentration data will be provided in a separate Clinical Pharmacology analysis plan.

12.3 Exploratory Endpoints

The exploratory endpoints are as follows:

The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study

The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study

12.3.1 Analysis on Exploratory Endpoints

Observed values and change from baseline for both ADHD-RS IV score and BRIEF score will be summarized at each visit. In Part 2, change in both scores from Week 73 visit will also be summarized for subjects transitioning from control to pegvaliase.

13 SAFETY EVALUATIONS

Safety will be assessed by examining the incidence, exposure adjusted event rate, severity grade, and relationship to study drug of all treatment-emergent adverse events (TEAEs) reported during the study period. In addition, clinical laboratory results, vital signs, pregnancy test, physical exam and ECG values will be assessed. In general, safety assessments will be summarized by treatment received in Part 1 (pegvaliase and diet-only) and in Part 1 and 2 (pegvaliase and diet-only).

13.1 Adverse Events

AEs will be coded in accordance with the MedDRA (version xx). Only treatment-emergent adverse events (TEAEs) occurring and reported during the study period will be included in adverse event summaries. A TEAE is defined as any AE that newly appeared or worsened in severity after first dose of pegvaliase (pegvaliase arm) or on or after study day 1 (diet-only arm) until 30 days after last dose of the study. If the onset date of an AE is missing, the AE will be considered treatment-emergent.

In Part 2 subjects transitioning from control to pegvaliase, a TEAE occurred during pegvaliase treatment period is defined as any AE that appeared or worsened in severity after first dose of pegvaliase until 30 days after last dose.

13.1.1 All Adverse Events

The incidence and frequency of all treatment-emergent AEs will be summarized by system organ class (SOC), preferred term (PT), relationship to study drug and National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) severity grades. For AEs that occurred more than once during the study, the maximum severity will be used to summarize the AEs by severity.

If the onset date or end date of an adverse event is partial, the same imputation rules described in Section 9 will be applied.

The following summary will be performed for treatment-emergent AEs:

- All AEs by SOC and PT
- All AEs by SOC, PT and severity
- AEs assessed by investigators as related to study drug by SOC and PT
- AEs assessed by investigators as related to study drug by SOC, PT and severity
- Serious AEs (SAEs) by SOC and PT

- All AEs by PT in descending frequency order
- All AEs by SOC and PT with exposure adjusted event rate
- AEs leading to withdrawal from study by SOC and PT
- AEs leading to study drug discontinuation by SOC and PT

In addition, summary tables and listings will be provided for AEs of special interest:

- Acute systemic hypersensitivity reaction (refer to Appendix 1~~Appendix 1~~)
- Anaphylaxis per FDA criteria (refer to Appendix 1~~Appendix 1~~)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

13.2 Clinical Laboratory Tests

Descriptive statistics will be presented for clinical laboratory tests at each scheduled visit and for change in laboratory values from baseline. The proportion of subjects who experienced at least one laboratory test result outside the normal ranges will be presented for each measurement as appropriate. Shift table from baseline to most extreme post-baseline value based on normal range and based on CTC grading (where available) will also be generated for each parameter as appropriate. A subject listing of laboratory test results for hematology, chemistry, urinalysis, and other laboratory tests will be provided separately. Values with CTC grade as 3 or above will be flagged in the listings.

A listing of laboratory test results for subjects with alanine transaminase (ALT) or alanine aminotransferase (AST) ≥ 3xULN and total bilirubin ≥ 2xULN will also be provided.

13.3 Vital Signs

Vital signs will include systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

Summary statistics including n, mean, SD, median, minimum, and maximum will be provided for these parameters at each visit. A subject listing will also be provided.

13.4 12-lead Electrocardiogram

The frequency of abnormalities and clinically significant abnormalities in 12-lead electrocardiogram will be summarized at each visit. A subject listing of ECG findings will be provided.

13.5 Physical Examination

Physical examinations will include assessments of general appearance; head, eyes, ears, nose, and throat; the cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Other body systems may be examined if needed. A subject listing for clinically significant findings at each visit will be provided.

13.6 Immunogenicity Tests

Immunogenicity will be performed using validated immunogenicity assays. Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). In the event of a Hypersensitivity Reaction visit (HRV), anti-pegvaliase IgE will be assessed.

For immunogenicity analysis, incidence tables and titer summary statistics including mean, median, standard deviation, and minimum/maximum titer values at each study visit will be provided for each antibody analyte.

Anti-drug antibody impact on safety will be evaluated. Plots and tables will be generated to explore the potential impact of anti-drug antibodies on HAE frequency and severity. A listing will be provided for each subject who has Hypersensitivity Reaction Visit.

Anti-drug antibody impact on efficacy will be evaluated. Plots and tables may be generated to explore the potential impact of anti-drug antibodies on blood Phe levels.

13.7 Pregnancy Test

A subject listing for pregnancy urine sample test at each visit will be provided.

14 APPENDICES

Appendix 1: Search Strategies for Adverse Event of Special Interest, and Sponsor Defined AEs of Clinical Significance

MedDRA version: most current version at program termination

1. Acute systemic hypersensitivity reaction

An anaphylaxis episode which meets the NIAID/FAAN criteria and is positively adjudicated by an independent expert allergist/immunologist as anaphylaxis according to the NIAID/FAAN criteria and based on clinical judgment of the significance of symptoms, is referred to as an acute systemic hypersensitivity reaction.

2. Anaphylaxis per FDA criteria

Anaphylaxis events defined according to the DPARP methodology are referred to as "anaphylaxis per FDA criteria". The FDA's assessment methodology includes individual case safety reports with any of the following characteristics:

Clinical manifestations consistent with NIAID/FAAN Criterion 1 (skin involvement + respiratory or cardiovascular compromise); and

Verbatim reports of anaphylaxis or anaphylactic reaction, even if NIAID/FAAN criteria were not met; and

Cases involving subject injection of epinephrine alone, even if subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

Anaphylaxis episodes meeting these FDA criteria are the primary focus of the safety assessment.

3. Angioedema per sponsor criteria

A 2-step process to identify angioedema per sponsor criteria, the following assessment was performed on the entire AE dataset:

- Step 1: all reported ADRs suggestive of angioedema using the Angioedema MedDRA HLT search, which includes the following 21 PTs: angioedema, circumoral oedema, eyelid oedema, face oedema, Gleich's syndrome, hereditary angioedema, idiopathic angioedema, intestinal angioedema, laryngeal oedema, laryngotracheal oedema, lip oedema, lip swelling, mouth swelling, oculorespiratory syndrome, oedema mouth, oropharyngeal swelling, periorbital oedema, pharyngeal oedema, swelling face, swollen tongue, and tongue oedema.
- Step 2: BioMarin then clinically assessed the retrieved data identified from Step 1 to assign concurrent events into episodes and to exclude:
  - False positive ADR reports that have clear confounders which impact assessment, eg, onset of event(s) ≥ 30 days after last dose or events/episodes with a very clear alternative etiology, such as throat swelling secondary to intubation). Cases were not excluded where the events have a long duration which may be atypical for angioedema, or where events occurred concurrent with other conditions which may have contributed to their development (eg, eye swelling reported during episode(s) of seasonal allergy).
- Episodes which occurred as part of an acute systemic hypersensitivity reaction and, therefore, will already be included in the incidence for acute systemic hypersensitivity reactions (to avoid duplication).

In the sponsor's opinion, the methodology described above is more likely to be reflective of the true incidence of angioedema manifestations.

4. Serum Sickness

Serum sickness-like reaction will be defined by including all preferred term of serum sickness, serum sickness-like reactions and Type III immune complex mediated reactions.

5. Severe Injection-site reaction

Severe injection site reaction will be identified as any injection site reaction report that is reported as Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or above in severity and meeting MedDRA high level term (HLT) for Injection site reaction, or is reported as a Serious Adverse Reaction, irrespective of severity.

6. Hypophenylalanemia

Hypophenylalaninemia is defined as a blood phenylalanine (Phe) level ≤ 30 µmol/L for a minimum of 2 consecutive values, with the start of the event defined as the first blood Phe assessment day with < 30 µmol/L and the end of the event defined as the day prior to the first blood Phe assessment ≥ 30 µmol/L

7. Persistent arthralgia (> 6 months)

Arthralgia will be identified as the preferred terms of arthralgia, pain in extremity, back pain, musculoskeletal pain, and neck pain. To be included in the results, the duration of the event should be > 6 months (Multiple AE records with same reported term and ≤ 1 day difference between the start date and end date are considered as same event).

105 Digital Drive

Novato, CA 94949

Statistical Analysis Plan, Version 1.0

Study 165-306

5 May 2022

1 APPROVALS (SIGNATURE AND DATE)

Title:	A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design
Protocol:	165-306 Amendment 1, 26Jan2022
Date:	May 5, 2022

{Please See Appended Electronic Signature Page}

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2 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
C3	complement component 3
C4	complement component 4
CRP	C-reactive protein
CRF	Case Report Form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	diastolic blood pressure
ECG	electrocardiogram
HAE	hypersensitivity adverse event
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
ITT	intent-to-treat
IWRS	Interactive Web Response System
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
Nab	neutralizing antibody
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
PAL	Phenylalanine ammonia-lyase
PEG	polyethylene glycol
Phe	phenylalanine
PK	pharmacokinetics
PKU	phenylketonuria
PT	preferred term
SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation

SOC	system organ class
SMQ	Standardized MedDRA Queries
TEAE	treatment-emergent adverse event
Tab	total antibody
WHO Drug	World Health Organization Drug Dictionary

3 INTRODUCTION

Study 306 is an open-label, two-arm (Active vs Diet-only Control), randomized control study to evaluate safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent subjects with phenylketonuria (PKU).

The purpose of this Statistical Analysis Plan (SAP) is to provide a comprehensive description of methods of the data analyses outlined in the protocol (amendment 1 dated 26 January 2022).

If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the SAP will prevail.

3.1 Objectives of Study

The primary objective of the study is:

- To evaluate the efficacy of pegvaliase in adolescent subjects with PKU
- To evaluate the safety of pegvaliase in adolescent subjects with PKU

The secondary objectives of the study are:

- To characterize total dietary protein intake in adolescent subjects with PKU after pegvaliase treatment
- To characterize the pharmacokinetics (PK) of pegvaliase in adolescent subjects with PKU

The exploratory objectives of the study are:

- To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent subjects with PKU
- To compare subjects' optimal maintenance pegvaliase dose across different age groups within the study
- Explore the biochemical, molecular, and cellular aspects of PKU
- PAH genotyping

3.2 Study Design

This is an open-label, two arm, randomized control study. Approximately 54 US and EU subjects (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with diet-only control will be enrolled. At enrollment, subjects will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with 36 subjects receiving daily pegvaliase and 18 subjects managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-in blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L.
Subjects in the active and control arms will follow the same visit schedules and perform the

same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control subjects will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, subjects will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, subjects may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), subjects will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for subjects in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of an Induction/Titration Dosing (9 weeks) and a Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40 or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (subjects on pegvaliase treatment only), and safety assessments.

Beginning Week 73, subjects in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 80 additional weeks (through Week 153). Beginning Week 73, subjects in the control treatment arm will initiate pegvaliase treatment and follow a parallel schedule for dosing, study visits, and assessments in Weeks 73 through 145 to that followed by the active arm subjects during the Primary Treatment Phase (Part 1) from Weeks 1 through 73, after which they will receive pegvaliase for up to 8 additional weeks (through Week 153).

3.3 Study Population

Individuals with PKU aged 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive with diet-only control at the start of the Screening/Run-in Period (Day -28) are candidates for participation in the study. Additional criteria for study participation are presented in Protocol 165-306 Section 5.1 and Section 5.2.

3.4 Study Dosage and Administration

Subjects will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

3.5 Sample Size Determination

Approximately 54 subjects will be enrolled and randomized to the pegvaliase or diet-only groups in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 subjects will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided).

Approximately 72 subjects will be screened for study participation such that approximately 54 subjects will be enrolled in the study.

For the pegvaliase treatment arm, the mean (SD) assumption of reduction in blood Phe is based on subjects 16 to 24 years of age who completed 17 months of treatment in previous pegvaliase studies utilizing an I/T/M dosing regimen (n = 46). For the diet-only arm, the mean (SD) assumption of reduction in blood Phe is based on BioMarin's PKUDOS (Phenylketonuria Demographic, Outcomes, and Safety) registry for patients aged 12 to <18 years old on diet only control with baseline Phe > 600 µmol/L at year 1 of study enrollment (n = 57).

3.6 Blinding and Randomization Methods

3.6.1 Blinding Method

Subjects will receive open-label pegvaliase or diet-only treatment.

3.6.2 Randomization Method

Subjects will be randomized in a 2:1 ratio to the active (pegvaliase) or control (diet-only) treatment arm, respectively. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L.

3.7 Interim Analysis

There are no interim analyses planned. The primary analysis will be performed after all subjects complete Part 1 or discontinued from the study. The final analyses will be performed after all subjects have completed the study or discontinued from the study.

4 GENERAL ANALYSIS CONSIDERATION

Descriptive summaries of continuous variables will include the mean, standard deviation (SD), median, minimum, and maximum. Descriptive summaries of categorical variables will include n, frequency, and percentage.

4.1 Analysis Populations

The All Enrolled population will consist of all subjects (or their parent/guardians, if applicable) who agree to participate in the clinical study following completion of the informed consent process and determination of study eligibility.

The Intent to Treat (ITT) population will consist of all subjects who are randomized. Subjects will be analyzed according to the randomized treatment.

The Safety population will consist of all subjects who received study treatment (pegvaliase or diet-only management of PKU). Subjects will be analyzed according to the treatment actually received during Part 1.

The Per Protocol (PP) population will consist of all subjects in the ITT population who are compliant with the protocol with no major protocol deviations impacting the efficacy analysis. The PP population will be determined by the study team through data review before database lock for primary analysis; reasons for excluding subjects will be defined and documented prior to database lock for primary analysis.

The PK population will consist of all subjects with at least one PK measurement.

4.2 Treatment Group Presentation

The following treatment groups will be used in summary tables:

Part 1: pegvaliase (active) or diet-only (control)

Part 2: pegvaliase (subjects continuing on pegvaliase) or diet-only (subjects transitioning from control to pegvaliase)

4.3 Pooling of Data from Sites with Small Enrollment

Since the enrollment for each site is expected to be small, the analyses will not be adjusted by site or pooled sites.

4.4 Study Day Derivation

Study days will be derived as follows:

Pegvaliase arm: the first dose date will be assigned as Study Day 1

Diet only arm: the Visit 1 date will be assigned as Study Day 1

For visit occurs on or after Study Day 1, Study Day = visit date – day 1 date + 1

For visit occurs before Study Day 1, Study Day = visit date – day 1 date. There is no Study Day 0.

4.4.1 Baseline Definition

The baseline for all safety and efficacy endpoints (when applicable) is defined as the last non-missing measurement collected prior to the first pegvaliase dose (pegvaliase arm) or prior to or on Visit 1 date (diet-only arm), unless otherwise specified.

Baseline blood Phe concentration is defined as the average of 3 pre-treatment measurements collected between screening/Run-in and Day 1 pre-dose.

Baseline total protein intake is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in.

4.5 Visit Window for Analysis

All efficacy and safety data will be summarized by study week of assessment. An assessment for a subject will be classified according to the study day of the assessment where it falls within a window. The windows are designated for each scheduled week of visit and centered on a target day. If there are two or more assessments within a designated window, the assessment that is closest to the target day will be used for analyses. If the two closest assessments to the target day are equidistant from the target day, then for numerical variable, the average of the two assessments will be used for analyses. For categorical variables, the worst of the 2 equidistant measurements, indicating less treatment effect, will be used.

Table 4.5.1 lists the scheduled weeks of the assessments collected in this study and the corresponding range of treatment days (window) during which a visit may have occurred.

Table 4.5.1: Visit Time Windows for the Treatment Period

Randomization arm	Derived Visit	Target Day[a]	Window[b]
Pegvalise	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 63
	Week XX (11 ≤ XX ≤ 19, every two weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 6 to Day (XX–1)*7 + 7
	Week 21	Day 141	Day 134 to Day 154

Randomization arm	Derived Visit	Target Day[a]	Window[b]
	Week XX (XX ≥ 25, every four weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14
Diet-Only Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 - 2 to Day (XX – 1)*7 + 4
	Week 9	Day 57	Day 54 to Day 63
	Week XX (11 ≤ XX ≤ 19, every two weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 6 to Day (XX–1)*7 + 7
	Week 21	Day 141	Day 134 to Day 154
	Week XX (13 ≤ XX ≤ 69, every four weeks)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 -13 to Day (XX -1)*7 + 14
	Week 73	Day 505	Day 491 to Day 508
Diet-Only Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3
	Week 81	Week 73 day + 56	Week 73 day + 53 to Week 73 day + 62
	Week XX (83 ≤ XX ≤ 91, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 7 to Week 73 day + (XX - 73)*7 + 6
	Week 93	Week 73 day + 56	Week 73 day + 133 to Week 73 day + 153
	Week XX (XX > 81, every four weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 14 to Week 73 day + (XX - 73)*7 + 13

[a] Target days are relative to the Study Day 1.

[b] Visit day is calculated by (visit date – Study Day 1 + 1).

Diet only group: all Part 2 visit is relative to the first dose day of pegvaliase

4.6 Handling of Dropouts and Missing Data

Subjects who discontinued prematurely will not be replaced.

Missing dates or partially missing dates will be imputed conservatively for concomitant medications and AEs to ensure that an AE is considered treatment emergent and has the longest possible duration. Missing data in blood Phe will be handled by multiple imputation method, more details are provided in Section 13.1.1

5 SUBJECT DISPOSITION

The total number of enrolled subjects, the number (%) of subjects who received study treatment, the number (%) of subjects completed Part 1, the number (%) of subjects who completed the study, and the number of subjects in each analysis population (ITT, Safety, PP and PK), as appropriate, will be summarized.

6 DISCONTINUATION AND COMPLETION

For subjects who prematurely discontinue the study, the primary reason for discontinuation will be summarized by treatment group. A similar summary will be provided for subjects who discontinue study treatment. A subject listing of completion and early termination from study will also be provided.

7 PROTOCOL DEVIATIONS

Both important and non-important protocol deviations will also be summarized by deviation category. All protocol deviations will be listed. Subjects with inclusion or exclusion criteria deviations will also be provided in a separate listing.

8 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Subject demographic and baseline characteristics will be summarized for the ITT population. Demographics include age, age category (12-13, 14-15 and 16-17 years old), gender, race, country and ethnicity. Baseline characteristics include height, weight, BMI, blood Phe concentration, neurocognitive assessment, three-day average dietary intake, restricted diet, genotyping and antibody status.

9 MEDICAL HISTORY

Each subject's chronic diseases, disorders, and surgeries in the past will be collected as general medical history. General medical history will be summarized by System Organ Class (SOC) and Preferred Term (PT) by descending order of frequency. A by-subject listing of each subject's medical history will also be provided.

10 PRIOR AND CONCOMITANT MEDICATIONS/PROCEDURES

The following definitions will be used to determine prior and concomitant medications:

- Prior medications: any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Concomitant medications: any medications initially taken on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Prior and concomitant medications: any medications taken both prior to and on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) will be reported both as prior and concomitant medications. Therefore, any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) where the stop date is reported as "continuing" or missing will be considered as both prior and concomitant medications.

In the event the start date of a medication is partial, the following imputation rules will be applied:

If only day is missing, then the start date will be imputed as the first day of the month, if the year and month is same or after the first dose year and month. If only day is missing, then the start date will be imputed as the last day of the month, if the year and month is prior to the first dose year and month. If month is the same as the month of study day 1 then the start date will be imputed as the date of study day 1.

- If only year is non-missing, then the start date will be imputed as the first day of the year. If year is the same as the year of study day 1 then the start date will be imputed as the date of study day 1.

In the event the stop date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the end date will be imputed as the last day of the month.
- If only year is non-missing, then the end date will be imputed as the last day of the year.

All medications will be coded using the current version of the World Health Organization Drug (WHO Drug) dictionary (MMM YYYY). Prior and concomitant medication use will be separately summarized by Anatomical Therapeutic Chemical (ATC) medication class (Level 4) and preferred name (i.e., generic medication name). A subject reporting the same medication use more than once will be counted once when calculating the number and percentage of subjects who received that medication. A subject listing of each subject's prior and concomitant medications will be provided.

11 STUDY DRUG USAGE

Study drug usage rate will be calculated based on the drug exposure records captured in eCRFs and dispensed drug amount captured in the Interactive web response system (IWRS).

The overall study drug usage rate will be derived from the total amount of study drug intake divided by the total dispensed study drug amount over the study period and multiplied by 100%. The rate will be calculated for Part 1, Part 2 and overall study period.

12 EXTENT OF EXPOSURE TO STUDY TREATMENT

For pegvaliase treatment period, the total number of days on study treatment, total amount of study drug (mg) taken and average daily dose (mg) will be summarized for Part 1, Part 2 and overall study period. Any dose interruption longer than 28 days will be excluded from the total exposure duration calculation.

In addition, the total number of days on diet control treatment will be summarized for pegvaliase and diet-only arm, the summary of protein intake is described in Section 13.2.1

A by-subject listing of each subject's extent of exposure and compliance will be provided.

13 EFFICACY EVALUATIONS

Unless otherwise specified, efficacy analysis will be based on the ITT population.

13.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study, which is defined as the average of Week 69 and Week 73 blood Phe measurements – baseline blood Phe. Change from baseline in blood Phe will be compared between subjects in the pegvaliase arm and the diet-only arm.

13.1.1 Primary Estimand

Population: the target study population comprises adolescent subjects (ages 12-17) with PKU who meet the inclusion and exclusion criteria as specified in the protocol. The analysis population is the ITT population as defined in Section 4.1

Variable: the variable is the primary efficacy endpoint, change from baseline in blood Phe following 72 weeks on study, as defined in Section 13.1.

Handling of missing data and intercurrent events (ICEs): the treatment policy will be adopted which will include all blood Phe data regardless of intercurrent events such as treatment discontinuation. Missing blood Phe data will be imputed by multiple imputation methods as described in Section 13.1.2.

Population level summary: the difference in change from baseline in blood Phe following 72 weeks on study will be estimated by an Analysis of Covariance (ANCOVA) model with multiple imputation of missing data, as described in Section 13.1.2.

13.1.2 Primary Analysis

The primary efficacy endpoint will be evaluated using an ANCOVA model, which will include baseline blood Phe as covariates and treatment group as factor, with missing data handled by multiple imputation. The imputation will be performed using the SAS procedure PROC MI; the variable list for imputation will include all baseline and post-baseline blood Phe measurements and stratified by treatment. The MCMC method will be used to generate 100 imputation datasets. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. The primary analysis will be based on the ITT population.

Descriptive summary of observed values, change and percent change from baseline in blood Phe will be provided by visit and treatment. In addition, categorical summary will be performed for the following categories:

In Part 2, change and percent change in blood Phe from average of Week 69 and Week 73 visits will also be summarized for subjects transitioning from control to pegvaliase.

13.1.3 Sensitivity Analysis

The same statistical model used in the primary analysis will be repeated in the PP population.

In addition, the primary analysis model (ANCOVA) will be repeated on the ITT population using 1) observed data only and 2) observed data and missing data imputed by Last Observation Carried Forward (LOCF).

13.2 Secondary Endpoints

The secondary endpoints are as follows:

Change from baseline in total dietary protein intake (i.e., medical food and/or intact food). The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits.

Plasma sample for trough PK

13.2.1 Analysis on Secondary Endpoints

For the change from baseline in total dietary protein intake, the observed values, change and percent change from baseline at each visit will be summarized by treatment group. Similar summary will also be provided for protein intake from medical food and from intact food separately. The values collected on the three-day diet diary will be averaged for each parameter at each visit before being summarized.

In Part 2, change and percent change in dietary protein intake from average of Week 69 and Week 73 visits will also be summarized for subjects transitioning from control to pegvaliase.

For plasma sample of trough PK, table summary and subject listing of observed value will be provided at each visit. Details of PK analysis of pegvaliase concentration data will be provided in a separate Clinical Pharmacology analysis plan.

13.3 Exploratory Endpoints

The exploratory endpoints are as follows:

The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study

The Behavior Rating Inventory of Executive Function (BRIEF) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study

Time to optimal maintenance dose by age groups (12-15 years and 16-17 years)

Number of patients who require the highest (60mg/day) maintenance dose

Percentage of patients in the blood Phe range of less than 600 µmol/L and less than 360 µmol/L, respectively

13.3.1 Analysis on Exploratory Endpoints

Descriptive summary will be provided for all exploratory endpoints by treatment group. Observed values and change from baseline for both ADHD-RS IV score and BRIEF score will be summarized at each visit. In Part 2, change in both scores from Week 73 visit will also be summarized for subjects transitioning from control to pegvaliase.

14 SAFETY EVALUATIONS

Safety will be assessed by examining the incidence, exposure adjusted event rate, severity grade, and relationship to study drug of all treatment-emergent adverse events (TEAEs) reported during the study period. In addition, clinical laboratory results, vital signs, pregnancy test, physical exam and ECG values will be assessed. In general, safety assessments will be summarized by treatment received in Part 1 (pegvaliase and diet-only) and in Part 1 and 2 (pegvaliase and diet-only).

14.1 Adverse Events

AEs will be coded in accordance with the MedDRA (version xx). Only treatment-emergent adverse events (TEAEs) occurring and reported during the study period will be included in adverse event summaries. A TEAE is defined as any AE that newly appeared or worsened in severity after first dose of pegvaliase (pegvaliase arm) or on or after study day 1 (diet-only arm) until 30 days after last dose of the study. If the onset date of an AE is missing, the AE will be considered treatment-emergent.

In Part 2 subjects transitioning from control to pegvaliase, a TEAE occurred during pegvaliase treatment period is defined as any AE that appeared or worsened in severity after first dose of pegvaliase until 30 days after last dose.

14.1.1 All Adverse Events

The incidence and frequency of all treatment-emergent AEs will be summarized by system organ class (SOC), preferred term (PT), relationship to study drug and National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) severity grades. For AEs that occurred more than once during the study, the maximum severity will be used to summarize the AEs by severity.

If the onset date or end date of an adverse event is partial, the same imputation rules described in Section 10 will be applied.

The following summary will be performed for treatment-emergent AEs:

- All AEs by SOC and PT
- All AEs by SOC, PT and severity
- AEs assessed by investigators as related to study drug by SOC and PT
- AEs assessed by investigators as related to study drug by SOC, PT and severity
- Serious AEs (SAEs) by SOC and PT
- All AEs by PT in descending frequency order

- All AEs by SOC and PT with exposure adjusted event rate
- AEs leading to withdrawal from study by SOC and PT
- AEs leading to study drug discontinuation by SOC and PT

In addition, summary tables and listings will be provided for AEs of special interest:

- Acute systemic hypersensitivity reaction (refer to Appendix 1)
- Anaphylaxis per FDA criteria (refer to Appendix 1)
- Angioedema
- Serum sickness
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration > 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)

14.2 Clinical Laboratory Tests

Descriptive statistics will be presented for clinical laboratory tests at each scheduled visit and for change in laboratory values from baseline. The proportion of subjects who experienced at least one laboratory test result outside the normal ranges will be presented for each measurement as appropriate. Shift table from baseline to most extreme post-baseline value based on normal range and based on CTC grading (where available) will also be generated for each parameter as appropriate. A subject listing of laboratory test results for hematology, chemistry, urinalysis, and other laboratory tests will be provided separately. Values with CTC grade as 3 or above will be flagged in the listings.

A listing of laboratory test results for subjects with alanine transaminase (ALT) or alanine aminotransferase (AST) ≥ 3xULN and total bilirubin ≥ 2xULN will also be provided.

14.3 Vital Signs

Vital signs will include systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

Summary statistics including n, mean, SD, median, minimum, and maximum will be provided for these parameters at each visit. A subject listing will also be provided.

14.4 12-lead Electrocardiogram

The frequency of abnormalities and clinically significant abnormalities in 12-lead electrocardiogram will be summarized at each visit. A subject listing of ECG findings will be provided.

14.5 Physical Examination

Physical examinations will include assessments of general appearance; head, eyes, ears, nose, and throat; the cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Other body systems may be examined if needed. A subject listing for clinically significant findings at each visit will be provided.

14.6 Immunogenicity Tests

Immunogenicity will be performed using validated immunogenicity assays. Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). In the event of a Hypersensitivity Reaction visit (HRV), anti-pegvaliase IgE will be assessed.

For immunogenicity analysis, incidence tables and titer summary statistics including mean, median, standard deviation, and minimum/maximum titer values at each study visit will be provided for each antibody analyte.

Anti-drug antibody impact on safety will be evaluated. Plots and tables will be generated to explore the potential impact of anti-drug antibodies on HAE frequency and severity. A listing will be provided for each subject who has Hypersensitivity Reaction Visit.

Anti-drug antibody impact on efficacy will be evaluated. Plots and tables may be generated to explore the potential impact of anti-drug antibodies on blood Phe levels.

14.7 Pregnancy Test

A subject listing for pregnancy urine sample test at each visit will be provided.

15 APPENDICES

Appendix 1: Search Strategies for Adverse Event of Special Interest, and Sponsor Defined AEs of Clinical Significance

MedDRA version: most current version at program termination

1. Anaphylaxis per NIAID/FAAN criteria

Anaphylaxis per NIAID/FAAN is defined using the NIAID/FAAN clinical diagnostic criteria through review on the potential anaphylaxis per NIAID/FAAN cases, which are identified using Broad algorithmic anaphylactic reaction SMQ with the below algorithm:

Algorithm = A or (B and C) or (D and (B or C))

Name of Search	Category	Preferred Term
Broad Algorithmic Anaphylactic Reaction SMQ	A	Anaphylactic reaction
	A	Anaphylactic shock
	A	Anaphylactic transfusion reaction
	A	Anaphylactoid reaction
	A	Anaphylactoid shock
	A	Circulatory collapse
	A	Dialysis membrane reaction
	A	Kounis syndrome
	A	Procedural shock
	A	Shock
	A	Shock symptom
	A	Type I hypersensitivity
	B	Acute respiratory failure
	B	Asthma
	B	Bronchial oedema
	B	Bronchospasm
	B	Cardio-respiratory distress
	B	Chest discomfort
	B	Choking
	B	Choking sensation
	B	Circumoral oedema
	B	Cough
	B	Cough variant asthma

Name of Search	Category	Preferred Term
	B	Cyanosis
	B	Dyspnoea
	B	Hyperventilation
	B	Irregular breathing
	B	Laryngeal dyspnoea
	B	Laryngeal oedema
	B	Laryngospasm
	B	Laryngotracheal oedema
	B	Mouth swelling
	B	Nasal obstruction
	B	Oedema mouth
	B	Oropharyngeal oedema
	B	Oropharyngeal spasm
	B	Oropharyngeal swelling
	B	Pharyngeal oedema
	B	Pharyngeal swelling
	B	Respiratory arrest
	B	Respiratory distress
	B	Respiratory failure
	B	Reversible airways obstruction
	B	Sensation of foreign body
	B	Sneezing
	B	Stridor
	B	Swollen tongue
	B	Tachypnoea
	B	Throat tightness
	B	Tongue oedema
	B	Tracheal obstruction
	B	Tracheal oedema
	B	Upper airway obstruction
	B	Vaccine associated enhanced respiratory disease
	B	Wheezing
	C	Allergic oedema
	C	Angioedema
	C	Circumoral swelling
	C	Erythema
	C	Eye oedema

Name of Search	Category	Preferred Term
	C	Eye pruritus
	C	Eye swelling
	C	Eyelid oedema
	C	Face oedema
	C	Flushing
	C	Injection site urticaria
	C	Lip oedema
	C	Lip swelling
	C	Nodular rash
	C	Ocular hyperaemia
	C	Oedema
	C	Oedema blister
	C	Periorbital oedema
	C	Periorbital swelling
	C	Pruritus
	C	Pruritus allergic
	C	Rash
	C	Rash erythematous
	C	Rash pruritic
	C	Skin swelling
	C	Swelling
	C	Swelling face
	C	Swelling of eyelid
	C	Urticaria
	C	Urticaria papular
	D	Blood pressure decreased
	D	Blood pressure diastolic decreased
	D	Blood pressure systolic decreased
	D	Cardiac arrest
	D	Cardio-respiratory arrest
	D	Cardiovascular insufficiency
	D	Diastolic hypotension
	D	Hypotension
	D	Hypotensive crisis
	D	Post procedural hypotension

2. Acute systemic hypersensitivity reaction

An acute systemic hypersensitivity reaction (referred to as anaphylaxis in the protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board,

3. Anaphylaxis per FDA criteria

Anaphylaxis events defined according to the DPARP methodology are referred to as "anaphylaxis per FDA criteria". The FDA's assessment methodology includes individual case safety reports with any of the following characteristics:

- Clinical manifestations consistent with NIAID/FAAN Criterion #1 (skin involvement + respiratory or cardiovascular compromise); or
- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

4. Angioedema per sponsor criteria

A 2-step process to identify angioedema per sponsor criteria, the following assessment was performed on the entire AE dataset:

- Step 1: all reported ADRs suggestive of angioedema using the Angioedema MedDRA HLT search, which includes the following 21 PTs: angioedema, circumoral oedema, eyelid oedema, face oedema, Gleich's syndrome, hereditary angioedema, idiopathic angioedema, intestinal angioedema, laryngeal oedema, laryngotracheal oedema, lip oedema, lip swelling, mouth swelling, oculorespiratory syndrome, oedema mouth, oropharyngeal swelling, periorbital oedema, pharyngeal oedema, swelling face, swollen tongue, and tongue oedema.
- Step 2: BioMarin then clinically assessed the retrieved data identified from Step 1 to assign concurrent events into episodes and to exclude:
  - False positive ADR reports that have clear confounders which impact assessment, e.g., onset of event(s) $\geq$ 30 days after last dose or events/episodes with a very clear alternative etiology, such as throat swelling secondary to intubation). Cases were not excluded where the events have a long duration which may be atypical for angioedema, or where events occurred concurrent with other conditions which may have contributed to their development (e.g., eye swelling reported during episode(s) of seasonal allergy).
  - Episodes which occurred as part of an acute systemic hypersensitivity reaction and, therefore, will already be included in the incidence for acute systemic hypersensitivity reactions (to avoid duplication).

In the sponsor's opinion, the methodology described above is more likely to be reflective of the true incidence of angioedema manifestations.

5. Serum Sickness

Serum sickness-like reaction will be defined by including all preferred term of serum sickness, serum sickness-like reactions and Type III immune complex mediated reactions.

6. Severe Injection-site reaction

Severe injection site reaction will be identified as any injection site reaction report that is reported as Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or above in severity and meeting MedDRA high level term (HLT) for Injection site reaction.

7. Hypophenylalaninemia

Hypophenylalaninemia is defined as a blood phenylalanine (Phe) level $\leq$ 30 µmol/L for a minimum of 2 consecutive values, with the start of the event defined as the first blood Phe assessment day with < 30 µmol/L and the end of the event defined as the day prior to the first blood Phe assessment $\geq$ 30 µmol/L.

8. Severe or Persistent (> 6 months) arthralgia

Arthralgia will be identified as the preferred terms of arthralgia, pain in extremity, back pain, musculoskeletal pain, and neck pain. Persistent arthralgia is defined as any arthralgia event that has a duration of the event > 6 months (Multiple AE records with same reported term and ≤ 1 day difference between the start date and end date are considered as same event). Severe arthralgia is defined as any arthralgia event that is reported as CTCAE Grade 3 or above in severity.

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105 Digital Drive

Novato, CA 94949

Statistical Analysis Plan

Study 165-306

Version 1.1

2 October 2024

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1 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
C3	complement component 3
C4	complement component 4
CRP	C-reactive protein
CRF	Case Report Form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	diastolic blood pressure
ECG	electrocardiogram
HAE	hypersensitivity adverse event
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
ITT	intent-to-treat
IWRS	Interactive Web Response System
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
Nab	neutralizing antibody
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
PAL	Phenylalanine ammonia-lyase
PEG	polyethylene glycol
Phe	phenylalanine
PK	pharmacokinetics
PKU	phenylketonuria
PT	preferred term

SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
SOC	system organ class
SMQ	Standardized MedDRA Queries
TEAE	treatment-emergent adverse event
Tab	total antibody
WHO Drug	World Health Organization Drug Dictionary

2 INTRODUCTION

Study 165-306 is an open-label, two-arm (Active vs Diet-only Control), randomized control study to evaluate safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent participants with phenylketonuria (PKU).

The purpose of this Statistical Analysis Plan (SAP) is to provide a comprehensive description of methods of the data analyses outlined in the protocol (amendment 2 dated 23 October 2023).

If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the SAP will prevail. The discrepancies will be documented in the clinical study report (CSR).

2.1 Objectives of Study

The primary objective of the study is:

- To evaluate the efficacy of pegvaliase in adolescent participants with PKU
- To evaluate the safety of pegvaliase in adolescent participants with PKU

The secondary objectives of the study are:

- To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment
- To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU

The exploratory objectives of the study are:

- To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU
- To assess changes in PKU symptoms and impacts
- To compare participants' optimal maintenance pegvaliase dose across different age groups within the study
- Explore the biochemical, molecular, and cellular aspects of PKU
- PAH genotyping

2.2 Study Design

This is an open-label, two arm, randomized controlled study. Approximately 54 US and EU participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with diet-only control will be enrolled. At enrollment, participants will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with approximately 36 participants receiving daily pegvaliase and 18 participants managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-in blood Phe (mean of the 2 pre-

treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. Participants in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), participants will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for participants in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of an Induction/Titration Dosing (9 weeks) and a Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40 or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active participants only), and safety assessments.

Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 142 additional weeks (through Week 215). Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Weeks 73 through 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed for pegvaliase dosing starting from Study Day 1.

2.3 Study Population

Individuals with PKU aged 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive with diet-only control at the start of the Screening/Run-in Period (Day -28) are candidates for participation in the study. Additional criteria for study participation are presented in Protocol 165-306 Section 5.1 and Section 5.2.

2.4 Study Dosage and Administration

Participants will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum

dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

2.5 Sample Size Determination

Approximately 54 participants will be enrolled and randomized to the pegvaliase or diet-only groups in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided).

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

For the pegvaliase treatment arm, the mean (SD) assumption of reduction in blood Phe is based on participants 16 to 24 years of age who completed 17 months of treatment in previous pegvaliase studies utilizing an I/T/M dosing regimen (n = 46). For the diet-only arm, the mean (SD) assumption of reduction in blood Phe is based on BioMarin's PKUDOS (Phenylketonuria Demographic, Outcomes, and Safety) registry for patients aged 12 to <18 years old on diet only control with baseline Phe > 600 µmol/L at year 1 of study enrollment (n = 57).

2.6 Blinding and Randomization Methods

2.6.1 Blinding Method

Participants will receive open-label pegvaliase or diet-only treatment.

2.6.2 Randomization Method

Participants will be randomized in a 2:1 ratio to the active (pegvaliase) or control (diet-only) treatment arm, respectively. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L.

2.7 Interim Analysis

There are no interim analyses planned. The primary analysis will be performed after all participants complete Part 1 or discontinued from the study. The final analyses will be performed after all participants have completed the study or discontinued from the study.

3 GENERAL ANALYSIS CONSIDERATION

Descriptive summaries of continuous variables will include n, mean, standard deviation (SD), median, minimum, and maximum. Descriptive summaries of categorical variables will include n, frequency, and percentage.

3.1 Analysis Populations

The All Enrolled population will consist of all participants who agree to participate in the clinical study (by their parent/guardians, if applicable) following completion of the informed consent process and determination of study eligibility.

The Intent to Treat (ITT) population will consist of all participants who are randomized. Participants will be analyzed according to the randomized treatment for the analysis of efficacy.

The Safety population will consist of all participants who received at least one dose of study treatment (pegvaliase or diet-only management of PKU). Subjects will be analyzed according to the treatment actually received for the analysis of safety.

The Per Protocol (PP) population will consist of all participants in the ITT population who are compliant with the protocol with no important protocol deviations impacting the primary efficacy analysis. The PP population will be determined by the study team through data review before database snapshot for primary analysis; reasons for excluding participants will be defined and documented prior to database snapshot for primary analysis.

The PK population will consist of all participants who received pegvaliase with at least one PK measurement.

3.2 Treatment Group Presentation

The following treatment groups will be used in summary tables:

Part 1: pegvaliase (active) or diet-only (control)

Part 2: pegvaliase (participants continuing on pegvaliase) or diet-only (participants transitioning from control to pegvaliase)

3.3 Pooling of Data from Sites with Small Enrollment

Since the enrollment for each site is expected to be small, the analyses will not be performed by site or pooled sites.

3.4 Study Day Derivation

Study days will be derived as follows:

Pegvaliase arm: the first dose date will be assigned as Study Day 1

Diet only arm: the Visit 1 date will be assigned as Study Day 1

For visit occurs on or after Study Day 1, Study Day = visit date – day 1 date + 1

For visit occurs before Study Day 1, Study Day = visit date – day 1 date. There is no Study Day 0.

3.4.1 Baseline Definition

The baseline for all safety and efficacy endpoints (when applicable) is defined as the last non-missing measurement collected prior to the first pegvaliase dose (pegvaliase arm) or prior to or on Visit 1 date (diet-only arm), unless otherwise specified:

Baseline blood Phe concentration at Part 1 is defined as the average of 3 pre-treatment measurements collected between screening/Run-in (2) and Day 1 pre-dose (1). Baseline blood Phe concentration at Part 2 is defined as the average of week 69 and week 73 measurements.

Baseline total protein intake at Part 1 is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in (2) and Day 1 (1). Baseline total protein intake at Part 2 is defined as the average of week 69 and week 73 measurements

For other measurements,

- Pegvaliase:
    - Baseline for the whole study period: last non-missing measurement prior to first pegvaliase dose
- Diet only:
    - Baseline for Part 1: last non-missing measurement prior to visit day 1
    - Baseline for Part 2: measurements taken prior to first dose of pegvaliase at Week 73

3.4.2 Treatment Period and Phase

Pegvaliase treatment period is defined from study Day 1 until 30 days after last dose.

Definitions of Induction, titration and maintenance phase during pegvaliase treatment period are as follows:

Induction: When subjects are on a 2.5 mg weekly dose.

Titration: When subjects have completed the induction phase and begin to increase dose and dosing frequency until they meet the criterion for the Maintenance Phase.

Induction/Titration: The treatment period prior to the start of Maintenance Phase.

Maintenance (first occurrence):

- US: Completion of an 8-week period with at least 80% compliance at the same dose. The start of maintenance phase is at the end of the 8-week period.
- EU: when subjects have at least a 26-day period with all blood Phe assessments $\leq$ 600 µmol/L and are on stable dose, which is defined as on same dose with at least 80% compliance rate. The start of maintenance phase is the date of the first Phe assessment at which the above criteria are met.

Diet-only period is defined from study Day 1 until the day before first dose of pegvailiase or 30 days after early termination, whichever occurs earlier.

3.5 Visit Window for Analysis

All efficacy and safety data will be summarized by study week of assessment. An assessment for a subject will be classified according to the study day of the assessment where it falls within a window. The windows are designated for each scheduled week of visit and centered on a target day. If there are two or more assessments within a designated window, the assessment that is closest to the target day will be used for analyses. If the two closest assessments to the target day are equidistant from the target day, then for numerical variable, the average of the two assessments will be used for analyses. For categorical variables, the worst of the 2 equidistant measurements, indicating less treatment effect, will be used.

Table 3.5.1 lists the scheduled weeks of the assessments collected in this study and the corresponding range of treatment days (window) during which a visit may have occurred.

Table 3.5.1: Visit Time Windows for the Treatment Period

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 63
	Week XX (11 ≤ XX ≤ 19, every two weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 6 to Day (XX–1)*7 + 7
	Week 21	Day 141	Day 134 to Day 154
	Week XX (25≤XX≤209, every four weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14
	Week 213	Day 1485	Day 1471 to Day 1491
	Week 215	Day 1499	≥ Day 1492

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 - 2 to Day (XX – 1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤ XX ≤ 69, every four weeks)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 -13 to Day (XX -1)*7 + 14
	Week 73	Day 505	Day 491 to Day of first dose of pegvaliase
Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3
	Week 81	Week 73 day + 56	Week 73 day + 53 to Week 73 day + 62

	Week XX (83 ≤ XX ≤ 91, every two weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 7 to Week 73 day + (XX - 73)*7 + 6
	Week 93	Week 73 day + 140	Week 73 day + 133 to Week 73 day + 153
	Week XX (97≤XX ≤ 209, every four weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 14 to Week 73 day + (XX - 73)*7 + 13
	Week 213	Day 1485	Week 73 day + 966 to Week 73 day + 986
	Week 215	Day 1499	≥ Week 73 day + 987

[a] Target days are relative to the Study Day 1.
[b] Visit day is calculated by (visit date – Study Day 1 + 1).
Diet only group: all Part 2 visit is relative to the first dose day of pegvaliase

3.6 Handling of Dropouts and Missing Data

Participants who discontinued prematurely will not be replaced.

Missing dates or partially missing dates will be imputed conservatively for concomitant medications and AEs to ensure that an AE is considered treatment emergent when possible and has the longest possible duration. If the onset date of an AE is completely missing, the AE will be considered treatment-emergent. Missing data in blood Phe will be handled by multiple imputation method, more details are provided in Section 12.1.1

4 SUBJECT DISPOSITION

The total number of enrolled participants, the number (%) of participants who received study treatment, the number (%) of participants completed Part 1, the number (%) of participants who completed the study, and the number of participants in each analysis population (ITT, Safety, PP and PK), as appropriate, will be summarized.

5 DISCONTINUATION AND COMPLETION

For participants who prematurely discontinue the study, the primary reason for discontinuation will be summarized by treatment group. A similar summary will be provided for participants who discontinue study treatment. A subject listing of completion and early termination from study will also be provided.

6 PROTOCOL DEVIATIONS

Both major and minor protocol deviations will be summarized by deviation category. All protocol deviations will be listed. Participants with inclusion or exclusion criteria deviations will also be provided in a separate listing.

7 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Subject demographic and baseline characteristics will be summarized for the ITT population. Demographics include age, age category (12 to < 16 and 16 to < 18 years old), gender, race, country and ethnicity. Baseline characteristics include height, weight, BMI, blood Phe concentration, neurocognitive assessment, three-day average dietary intake, restricted diet, genotyping and antibody status.

8 MEDICAL HISTORY

Each subject's chronic diseases, disorders, and surgeries in the past will be collected as general medical history. General medical history will be summarized by System Organ Class (SOC) and Preferred Term (PT) by descending order of frequency. A by-subject listing of each subject's medical history will also be provided.

9 PRIOR AND CONCOMITANT MEDICATIONS/PROCEDURES

The following definitions will be used to determine prior and concomitant medications:

- Prior medications: any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Concomitant medications: any medications initially taken on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Prior and concomitant medications: any medications taken both prior to and on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) will be reported both as prior and concomitant medications. Therefore, any such medications where the stop date is reported, reported as "continuing", or missing will be considered as both prior and concomitant medications.

In the event the start date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the start date will be imputed as the first day of the month, if the year and month is same or after the study day 1 year and month. If only day is missing, then the start date will be imputed as the last day of the month, if the year and month is prior to the first dose year and month. If year and month are the same as

the year and month of study day 1 then the start date will be imputed as the date of study day 1.

- If only year is non-missing, then the start date will be imputed as the first day of the year if the year is after the year of study day 1. If year is the same as the year of study day 1 then the start date will be imputed as the date of study day 1. If year is before the year of study day 1 then start date will be imputed to the last date of the year.

In the event the stop date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the end date will be imputed as the last day of the month.
- If only year is non-missing, then the end date will be imputed as the last day of the year.

All medications will be coded using the current version of the World Health Organization Drug (WHO Drug) dictionary (September 2021). Prior and concomitant medication use will be separately summarized by Anatomical Therapeutic Chemical (ATC) medication class (Level 4) and preferred name (i.e., generic medication name). A subject reporting the same medication use more than once will be counted once when calculating the number and percentage of participants who received that medication. A subject listing of each subject's prior and concomitant medications will be provided.

10 STUDY DRUG USAGE

Study drug usage rate will be calculated based on the drug exposure records captured in eCRFs and dispensed drug amount captured in the Interactive web response system (IWRS).

The overall study drug usage rate will be derived from the total amount of study drug intake divided by the total dispensed study drug amount over the study period and multiplied by 100%. The rate will be calculated for Part 1, Part 2 and overall study period.

11 EXTENT OF EXPOSURE TO STUDY TREATMENT

For pegvaliase treatment period, the total number of days on study treatment, total amount of study drug (mg) taken and average daily dose (mg) will be summarized for Part 1, Part 2 and overall study period. Any dose interruption longer than 28 days will be excluded from the total exposure duration calculation.

In addition, the total number of days on diet control treatment period will be summarized for diet-only arm, the summary of protein intake is described in Section 12.2.1

A by-subject listing of each subject's extent of exposure and compliance will be provided.

Compliance for pegvaliase will be calculated by the total amount of study drug intake divided by the total amount of study drug dispensed, and multiplied by 100%

12 EFFICACY EVALUATIONS

Unless otherwise specified, efficacy analysis will be based on the ITT population.

12.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements. Change from baseline in blood Phe will be compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms.

12.1.1 Primary Estimand

Population: the target study population comprises adolescent participants (ages 12-17) with PKU who meet the inclusion and exclusion criteria as specified in the protocol. The analysis population is the ITT population as defined in Section 3.1

Variable: the variable is the primary efficacy endpoint, change from baseline in blood Phe following 72 weeks on study, as defined in Section 12.1.

Handling of missing data and intercurrent events (ICEs): the treatment policy strategy will be adopted which will include all blood Phe data regardless of intercurrent events such as treatment discontinuation. Missing blood Phe data will be imputed by multiple imputation methods as described in Section 12.1.2.

Population level summary: the difference in change from baseline in blood Phe following 72 weeks on study will be estimated by an Analysis of Covariance (ANCOVA) model with multiple imputation of missing data, as described in Section 12.1.2.

12.1.2 Primary Analysis

The primary efficacy endpoint will be evaluated using an ANCOVA model, which will include baseline blood Phe levels as covariates, randomization strata (baseline Phe $\leq$ 1000 µmol/L or > 1000 µmol/L) and treatment group as factors, with missing data handled by multiple imputation. The imputation will be performed using the SAS procedure PROC MI; the variable list for imputation will include all baseline and post-baseline blood Phe measurements and stratified by treatment. The MCMC method will be used to generate 100 imputation datasets. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. The primary analysis will be based on the ITT population.

Descriptive summary of observed values, change and percent change from baseline in blood Phe will be provided by visit and treatment

In Part 2, change and percent change in blood Phe from average of Week 69 and Week 73 visits will also be summarized for participants transitioning from control to pegvaliase by visits scheduled in Part 2.

12.1.3 Sensitivity Analysis

The same statistical model used in the primary analysis will be repeated in the PP population.

In addition, the primary analysis model (ANCOVA) will be repeated on the ITT population using 1) observed data only and 2) observed data and missing data imputed by Last Observation Carried Forward (LOCF).

12.2 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (i.e., medical food and/or intact food) following 72 weeks on study. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits. Total dietary protein is measured in grams/kilogram (g/kg).
- Plasma sample for trough PK

12.2.1 Analysis on Secondary Endpoints

For the change from baseline in total dietary protein intake, the observed values, change and percent change from baseline at each visit will be summarized by treatment group. Similar summary will also be provided for protein intake from medical food and from intact food separately. The values collected on the three-day diet diary will be averaged for each parameter at each visit before being summarized.

In Part 2, change and percent change in dietary protein intake from average of Week 69 and Week 73 visits will also be summarized for participants transitioning from control (diet-only) treatment arm to pegvaliase.

For plasma sample of trough PK, table summary and subject listing of observed value will be provided at each visit. The PK parameters derived from the intensive PK samples will also be summarized. Details of PK analysis of pegvaliase concentration data will be provided in a separate Clinical Pharmacology analysis plan.

12.3 Exploratory Endpoints

The exploratory endpoints are as follows:

- The ADHD Rating Scale IV inattention subscale (ADHD-RS IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study
- The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study
- Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores
- Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS])
- Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC]) Time to participants' optimal maintenance dose
- Number of patients who require the highest (60mg/day) maintenance dose
- Percentage of patients in the blood Phe ranges of less than 600 µmol/L and less than 360 µmol/L, respectively

12.3.1 Analysis on Exploratory Endpoints

12.3.1.1 The ADHD Rating Scale IV (ADHD RS-IV) Inattention Subscale

The ADHD RS-IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom.

ADHD-RS Inattention (IA) subscale is the sum of the 9 odd-numbered items.

ADHD-RS Hyperactivity-Impulsivity (HI) subscale is the sum of the 9 even-numbered items

ADHD-RS total score will be the sum of ADHD-RS IA and ADHD-RS HI

12.3.1.2 Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2)

The BRIEF2 is a 63-item measure in 8 non-overlapping clinical scales and 2 validity scales. It is designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The same parent/guardian must complete the BRIEF2 at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

Behavior Regulation Index (BRI), Emotion Regulation Index (ERI), Cognitive Regulation Index (CRI) and Global Executive Composite (GEC) will be summarized, and their definition are as follows:

BRI = Inhibit + Self-Monitor

ERI = Shift + Emotional Control

CRI = Initiate + Working Memory + Plan/Organize + Task-Monitor + Organization of Materials

GEC = BRI + ERI + CRI

12.3.1.3 PKU Symptom Severity and Impacts Scale (PKU-SSIS) v2.0 – Adolescent Version

The PKU Symptom Severity and Impact Scale (PKU-SSIS) was designed to evaluate neuropsychological symptoms and impacts in early-treated patients with PKU. The PKU-SSIS is comprised of 22 items that constitute 3 symptoms domains: 1) Emotional, Mood and Psychological; 2) (Neuro)cognitive, Executive, and Intellectual Function; 3) Physical Health. It also has 4 impact domains: 1) Social Relations; 2) Level of Independence; 3) General Wellbeing; and 4) Self-care (Quinn 2022). The instrument total score is compiled by summing the domain scores and converting to a percentage score (ie, Total score = (summed score/88)*100). Participants will complete the PKU-SSIS as indicated in the SoA.

12.3.1.4 Global Anchor Measures (Caregiver Global Impression of Severity [CaGIS], Caregiver Global Impression of Change [CaGIC], Patient Global Impression of Severity [PGIS], Patient Global Impression of Change [PGIC], and Clinician Global Impression of Change [CGIC]

Global impression measures for severity and change (or global anchors) are standard items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs.

Global impression of severity anchor measures (for Caregivers and Patients) rate the overall disease severity using a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." Global impression of change anchor measures (for Caregivers, Participants, and Clinicians) assess if there has been an improvement or change in clinical status using a 5-point Likert scale from "Much better" to "Much worse.".

Descriptive summary will be provided for all exploratory endpoints by treatment group. ADHD-RS IV, BRIEF2, PKU-SSIS, and global anchor measures will be summarized at respective scheduled visits. Scale/domain scores and total scores for ADHD-RS IV, BRIEF2, and PKU-SSIS will be reported by timepoint. Change from baseline for ADHD-RS IV scores BRIEF2 scores and PKU-SSIS scores (by scale/domain and total scores for all three measures) will be summarized at each visit. In addition, ANCOVA models will be performed, which will include baseline measurements (when available) as covariates, randomization strata (baseline Phe $\leq$ 1000 µmol/L or > 1000 µmol/L) and treatment group as factors. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. In Part 2, change scores from Week 73 visit will also be summarized for participants transitioning from control to pegvaliase.

12.4 Examination of Efficacy by Subgroups

The following subgroup analysis will be performed on the primary endpoint:

- Baseline phe: $\leq$ 1000 µmol/L or > 1000 µmol/L
- Sex
- Baseline Age (12 to < 16, 16 to < 18)
- Country

13 SAFETY EVALUATIONS

Safety will be assessed by examining the incidence, exposure adjusted event rate, severity grade, and relationship to study drug of all treatment-emergent adverse events (TEAEs) reported during the study period. In addition, clinical laboratory results, vital signs, pregnancy test, physical exam and ECG values will be assessed. In general, two sets of safety analyses will be performed: Part 1 period and combined Part 1 and 2 periods. AE data will be summarized by the actual treatment received at the time of AE onset (pegvaliase or diet-only).

13.1 Adverse Events

AEs will be coded in accordance with the MedDRA (version 24.1). Only treatment-emergent adverse events (TEAEs) occurring and reported during the study period will be included in adverse event summaries. A TEAE is defined as any AE that newly appeared or worsened in severity after first dose of pegvaliase (pegvaliase arm) or on or after study day 1 (diet-only arm) until 30 days after last dose of the study.

In Part 2 participants transitioning from control to pegvaliase, a TEAE occurred during pegvaliase treatment period is defined as any AE that appeared or worsened in severity after first dose of pegvaliase until 30 days after last dose.

13.1.1 All Adverse Events

The incidence and frequency of all treatment-emergent AEs will be summarized by system organ class (SOC), preferred term (PT), relationship to study drug and National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) severity grades. For AEs that occurred more than once during the study, the maximum severity will be used to summarize the AEs by severity.

If the onset date or end date of an adverse event is partial, the same imputation rules described in Section 2.6 will be applied.

The following summary will be performed for treatment-emergent AEs:

- All AEs by SOC and PT
- All AEs by SOC, PT and severity
- AEs assessed by investigators as related to study drug by SOC and PT
- AEs assessed by investigators as related to study drug by SOC, PT and severity
- Serious AEs (SAEs) by SOC and PT

- All AEs by PT in descending frequency order
- All AEs by SOC and PT with exposure adjusted event rate
- AEs leading to withdrawal from study by SOC and PT
- AEs leading to study drug discontinuation by SOC and PT

In addition, summary tables and listings will be provided for AEs of special interest:

- Acute systemic hypersensitivity reaction (refer to Appendix 1)
- Anaphylaxis per FDA criteria (refer to Appendix 1)
- Anaphylaxis per NIAID/FAAN criteria
- Angioedema per FDA criteria
- Angioedema per sponsor criteria
- Serum sickness-like reaction
- Hypersensitivity reaction (EU)
- Hypersensitivity reaction (US)
- Injection site reaction
- Injection site skin reaction ≥ 14 days duration
- Generalized skin reaction ≥ 14 days duration
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3
- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration ≥ 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)
- Arthralgia (sponsor-defined)
- Neuropsychiatric disorder
- Hypersensitivity AEs
- Arthralgia /Arthritis
- Lymphadenopathy ≥ 14 Days Duration
- Persistent Urticaria ≥ 14 Days Duration
- Infection and Infestations ≥ 30 Days Duration
- Ischemic Heart Disease

- Proteinuria/Albuminuria
- Hematuria
- Blood Creatinine Increased
- Renal Impairment
- Increased liver function test or Events Involving Hepatic Impairment
- Anemia
- Thrombocytopenia
- Hemolysis
- Myalgia
- Serositis
- Potential central nervous system vascular complications

13.2 Clinical Laboratory Tests

Descriptive statistics will be presented for clinical laboratory tests at each scheduled visit and for change in laboratory values from baseline. The proportion of participants who experienced at least one laboratory test result outside the normal ranges will be presented for each measurement as appropriate. Shift table from baseline to most extreme post-baseline value based on normal range and based on CTCAE grading (where available) will also be generated for each parameter as appropriate. A subject listing of laboratory test results for hematology, chemistry, urinalysis, and other laboratory tests will be provided separately. Values with CTCAE Grade ≥ 3 will be flagged in the listings.

A listing of laboratory test results for participants with alanine transaminase (ALT) or alanine aminotransferase (AST) $\geq$ 3x ULN and total bilirubin $\geq$ 2x ULN will also be provided.

13.3 Vital Signs

Vital signs will include systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

Summary statistics including n, mean, SD, median, minimum, and maximum will be provided for these parameters at each visit. A subject listing will also be provided.

13.4 12-lead Electrocardiogram

The frequency of abnormalities and clinically significant abnormalities in 12-lead electrocardiogram will be summarized at each visit. A subject listing of ECG findings will be provided.

13.5 Physical Examination

Physical examinations will include assessments of general appearance; head, eyes, ears, nose, and throat; the cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Other body systems may be examined if needed. A subject listing for clinically significant findings at each visit will be provided.

13.6 Pregnancy Test

A subject listing for pregnancy urine sample test at each visit will be provided.

14 IMMUNOGENICITY TESTS

Immunogenicity will be performed using validated immunogenicity assays. Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). In the event of a Hypersensitivity Reaction visit (HRV), anti-pegvaliase IgE will be assessed.

For immunogenicity analysis, incidence tables and titer summary statistics including mean, median, standard deviation, and minimum/maximum titer values at each study visit will be provided for each antibody analyte.

Anti-drug antibody impact on safety will be evaluated. Plots and tables will be generated to explore the potential impact of anti-drug antibodies on Hypersensitivity Reaction (US and EU) frequency and severity. A listing will be provided for each subject who has Hypersensitivity Reaction Visit.

Anti-drug antibody impact on efficacy will also be evaluated. Plots and tables may be generated to explore the potential impact of anti-drug antibodies on blood Phe levels.

15 APPENDICES

Appendix 1: Search Strategies for Adverse Event of Special Interest, and Sponsor Defined AEs of Clinical Significance

MedDRA version: most current version at program termination

1. Anaphylaxis per NIAID/FAAN criteria

Anaphylaxis per NIAID/FAAN is defined using the NIAID/FAAN clinical diagnostic criteria through review on the potential anaphylaxis per NIAID/FAAN cases, which are identified using Broad algorithmic anaphylactic reaction SMQ with the below algorithm:

Algorithm = A or (B and C) or (D and (B or C))

Name of Search	Category	Preferred Term
Broad Algorithmic Anaphylactic Reaction SMQ	A	Anaphylactic reaction
	A	Anaphylactic shock
	A	Anaphylactic transfusion reaction
	A	Anaphylactoid reaction
	A	Anaphylactoid shock
	A	Circulatory collapse
	A	Dialysis membrane reaction
	A	Kounis syndrome
	A	Procedural shock
	A	Shock
	A	Shock symptom
	A	Type I hypersensitivity
	B	Acute respiratory failure
	B	Asthma
	B	Bronchial oedema
	B	Bronchospasm
	B	Cardio-respiratory distress
	B	Chest discomfort
	B	Choking
	B	Choking sensation
	B	Circumoral oedema
	B	Cough
	B	Cough variant asthma

Name of Search	Category	Preferred Term
	B	Cyanosis
	B	Dyspnoea
	B	Hyperventilation
	B	Irregular breathing
	B	Laryngeal dyspnoea
	B	Laryngeal oedema
	B	Laryngospasm
	B	Laryngotracheal oedema
	B	Mouth swelling
	B	Nasal obstruction
	B	Oedema mouth
	B	Oropharyngeal oedema
	B	Oropharyngeal spasm
	B	Oropharyngeal swelling
	B	Pharyngeal oedema
	B	Pharyngeal swelling
	B	Respiratory arrest
	B	Respiratory distress
	B	Respiratory failure
	B	Reversible airways obstruction
	B	Sensation of foreign body
	B	Sneezing
	B	Stridor
	B	Swollen tongue
	B	Tachypnoea
	B	Throat tightness
	B	Tongue oedema
	B	Tracheal obstruction
	B	Tracheal oedema
	B	Upper airway obstruction
	B	Vaccine associated enhanced respiratory disease
	B	Wheezing
	C	Allergic oedema
	C	Angioedema
	C	Circumoral swelling

Name of Search	Category	Preferred Term
	C	Erythema
	C	Eye oedema
	C	Eye pruritus
	C	Eye swelling
	C	Eyelid oedema
	C	Face oedema
	C	Flushing
	C	Injection site urticaria
	C	Lip oedema
	C	Lip swelling
	C	Nodular rash
	C	Ocular hyperaemia
	C	Oedema
	C	Oedema blister
	C	Periorbital oedema
	C	Periorbital swelling
	C	Pruritus
	C	Pruritus allergic
	C	Rash
	C	Rash erythematous
	C	Rash pruritic
	C	Skin swelling
	C	Swelling
	C	Swelling face
	C	Swelling of eyelid
	C	Urticaria
	C	Urticaria papular
	D	Blood pressure decreased
	D	Blood pressure diastolic decreased
	D	Blood pressure systolic decreased
	D	Cardiac arrest
	D	Cardio-respiratory arrest
	D	Cardiovascular insufficiency
	D	Diastolic hypotension
	D	Hypotension
	D	Hypotensive crisis

Name of Search	Category	Preferred Term
	D	Post procedural hypotension

2. Anaphylaxis per FDA criteria

Anaphylaxis events defined according to the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) methodology are referred to as "anaphylaxis per FDA criteria". The FDA's assessment methodology includes individual case safety reports with any of the following characteristics:

- Clinical manifestations consistent with National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Criterion #1 (skin involvement + respiratory or cardiovascular compromise); or
- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

3. Acute systemic hypersensitivity reaction

An acute systemic hypersensitivity reaction (referred to as anaphylaxis in the protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The acute systemic hypersensitivity reaction events will also be presented if they meet Brown's severe criteria as determined by the Internal Safety Review Board.

4. Angioedema per FDA criteria

Angioedema as an AE reported as any PT in the MedDRA Angioedema Higher Level Term excluding idiopathic angioedema, Gleich's syndrome, and hereditary angioedema; and also excluding any AE which is part of an Anaphylaxis per FDA criteria episode.

5. Angioedema per sponsor criteria

A 2-step process to identify angioedema per sponsor criteria, the following assessment was performed on the entire AE dataset:

- Step 1: all reported ADRs suggestive of angioedema using the Angioedema MedDRA HLT search, which includes the following 21 PTs: angioedema, circumoral oedema, eyelid oedema, face oedema, Gleich's syndrome, hereditary

angioedema, idiopathic angioedema, intestinal angioedema, laryngeal oedema, laryngotracheal oedema, lip oedema, lip swelling, mouth swelling, oculorespiratory syndrome, oedema mouth, oropharyngeal swelling, periorbital oedema, pharyngeal oedema, swelling face, swollen tongue, and tongue oedema.

- Step 2: BioMarin then clinically assessed the retrieved data identified from Step 1 to assign concurrent events into episodes and to exclude:
  - False positive ADR reports that have clear confounders which impact assessment, e.g., onset of event(s) ≥ 30 days after last dose or events/episodes with a very clear alternative etiology, such as throat swelling secondary to intubation). Cases were not excluded where the events have a long duration which may be atypical for angioedema, or where events occurred concurrent with other conditions which may have contributed to their development (e.g., eye swelling reported during episode(s) of seasonal allergy).
- Episodes which occurred as part of an acute systemic hypersensitivity reaction and, therefore, will already be included in the incidence for acute systemic hypersensitivity reactions (to avoid duplication).

In the sponsor's opinion, the methodology described above is more likely to be reflective of the true incidence of angioedema manifestations.

6. Serum Sickness-like reaction

Serum sickness-like reaction will be defined by including all preferred term of serum sickness, serum sickness-like reactions and Type III immune complex mediated reactions.

7. Hypersensitivity Reaction (EU)

Hypersensitivity reactions are defined as the modified hypersensitivity SMQ (narrow SMQ) or an anaphylaxis event as described in **Error! Reference source not found.** - Listing 1 and adjudicated as per the Acute Systemic Hypersensitivity Reaction in section 10.5.2 in Protocol

The following terms should be excluded from the Modified Hypersensitivity (Narrow SMQ) search criteria:

injection site rash

injection site urticaria

8. Hypersensitivity Reaction (US)

Hypersensitivity reaction is defined as the modified hypersensitivity SMQ (narrow SMQ), plus the anaphylaxis adjudicated by FDA criteria.

The following terms should be excluded from the Modified Hypersensitivity (Narrow SMQ) search criteria:

injection site rash

injection site urticaria

anaphylactic reaction

anaphylactoid reaction

9. Injection site reaction

Injection-site reaction will be identified using injection site reaction Higher Level MedDRA term.

10. Severe Injection-site reaction

Severe injection site reaction will be identified as any injection site reaction report that is reported as Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or above in severity and meeting MedDRA high level term (HLT) for Injection site reaction.

11. Injection site skin reaction ≥ 14 days duration

Injection site skin reaction with ≥ 14 days duration will be identified by preferred term search using the list below. To be included as injection site skin reaction, the duration of the event needs to be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Injection site skin reaction	Injection site anaesthesia
	Injection site extravasation
	Injection site phlebitis
	Injection site movement impairment
	Injection site lymphadenopathy
	Injection site atrophy

Name of Search	Preferred Term
	Injection site cyst
	Injection site erosion
	Injection site fibrosis
	Injection site granuloma
	Injection site induration
	Injection site mass
	Injection site necrosis
	Injection site oedema
	Injection site ulcer
	Injection site hypersensitivity
	Injection site irritation
	Injection site discomfort
	Injection site dysaesthesia
	Injection site hyperaesthesia
	Injection site hypoaesthesia
	Injection site swelling
	Injection site calcification
	Injection site pustule
	Injection site nodule
	Injection site scar
	Injection site bruising
	Injection site haematoma
	Injection site haemorrhage
	Injection site hypertrophy
	Injection site pain
	Injection site paraesthesia
	Injection site pruritus
	Injection site urticaria
	Injection site papule
	Injection site macule
	Injection site vasculitis
	Injection site plaque
	Injection site discolouration
	Injection site injury
	Injection site abscess
	Injection site abscess sterile

Name of Search	Preferred Term
	Injection site dermatitis
	Injection site erythema
	Injection site infection
	Injection site inflammation
	Injection site rash
	Injection site reaction
	Injection site thrombosis
	Injection site vesicles
	Injection site ischaemia
	Injection site cellulitis
	Injection site discharge
	Injection site scab
	Injection site eczema
	Injection site recall reaction
	Injection site exfoliation

12. Generalized skin reaction ≥ 14 days duration

Generalized skin reaction ≥ 14 days duration will be identified in following ways:

- Preferred term search using below listing, or
- Broad vasculitis SMQ

To be included as generalized skin reaction, the duration of the event needs to be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Generalized Skin Reaction	Acute cutaneous lupus erythematosus
	Acute febrile neutrophilic dermatosis
	Acute generalised exanthematous pustulosis
	Angiodermatitis
	Angioedema
	Angular cheilitis
	Atypical fibroxanthoma
	Atypical lymphocytic lobular panniculitis
	Autoimmune dermatitis
	Benign neoplasm of skin

Blister
Blister infected
Blister rupture
Blood blister
Capillaritis
Carbuncle
Cellulitis
Cellulitis enterococcal
Cellulitis gangrenous
Cellulitis staphylococcal
Cellulitis streptococcal
Cheilitis
Cheilitis granulomatosa
Cheilosis
Chronic cutaneous lupus erythematosus
Chronic papillomatous dermatitis
Chronic pigmented purpura
Circumoral oedema
Coma blister
CREST syndrome
Cutaneous calcification
Cutaneous lupus erythematosus
Cutaneous vasculitis
Cutaneovisceral angiomatosis with thrombocytopenia
Dermatitis
Dermatitis allergic
Dermatitis atopic
Dermatitis bullous
Dermatitis exfoliative
Dermatitis exfoliative generalised
Dermatitis infected
Dermatitis psoriasiform
Drug eruption
Drug reaction with eosinophilia and systemic symptoms
Dry gangrene
Dry skin
Ecchymosis

Eczema
Eczema nummular
Eczema vesicular
Eosinophilic panniculitis
Epidermal necrosis
Epidermolysis
Epidermolysis bullosa
Eruptive pseudoangiomatosis
Erythema
Erythema elevatum diutinum
Erythema induratum
Erythema infectiosum
Erythema migrans
Erythema multiforme
Erythema nodosum
Excessive granulation tissue
Excoriation
Exfoliative rash
Eyelid oedema
Face oedema
Fixed eruption
Furuncle
Gangrene
Granuloma
Granuloma annulare
Granuloma skin
Granulomatous dermatitis
Haemorrhage subcutaneous
Haemorrhage subepidermal
Haemorrhagic urticaria
Haemorrhagic vasculitis
Hand dermatitis
Henoch-Schonlein purpura
Hypersensitivity vasculitis
Hypertrophic scar
Idiopathic angioedema
Idiopathic urticaria

Infected dermal cyst
Infected skin ulcer
Interstitial granulolmatous dermatitis
Itching scar
Jaundice
Keratolysis exfoliativa acquired
Laryngeal oedema
Laryngotracheal oedema
Lichenification
Lip blister
Lip oedema
Lip swelling
Lipoatrophy
Lipohypertrophy
Livedo reticularis
Lupus vulgaris
Lupus-like syndrome
Macule
Medical device site laceration
Morphoea
Mouth swelling
Mucocutaneous rash
Mucocutaneous ulceration
Necrobiosis lipoidica diabeticorum
Necrotising panniculitis
Neoplasm skin
Neurodermatitis
Nodular vasculitis
Oedema mouth
Oropharyngeal swelling
Osler's nodes
Overlap syndrome
Palisaded neutrophilic granulomatous dermatitis
Palmar erythema
Palpable purpura
Panniculitis
Panniculitis lobular

Papule
Pemphigoid
Pemphigus
Periorbital oedema
Perivascular dermatitis
Petechiae
Pharyngeal oedema
Pharyngeal swelling
Pigmentation disorder
Pruritus
Pruritus allergic
Pruritus generalised
Psoriasis
Psoriatic arthropathy
Purpura
Purpura fulminans
Pustular psoriasis
Pyoderma
Rash
Rash erythematous
Rash follicular
Rash generalised
Rash macular
Rash maculo-papular
Rash maculovesicular
Rash morbilliform
Rash papular
Rash papulosquamous
Rash pruritic
Rash pustular
Rash rubelliform
Rash vesicular
Recall phenomenon
Red man syndrome
Rheumatoid nodule
Scar
Sclerodactylia

Scleroderma
Scleroedema
Seborrhoea
Seborrhoeic dermatitis
Septal panniculitis
Skin atrophy
Skin disorder
Skin dystrophy
Skin erosion
Skin exfoliation
Skin fibrosis
Skin haemorrhage
Skin hyperpigmentation
Skin hyperplasia
Skin hypertrophy
Skin hypopigmentation
Skin hypoplasia
Skin induration
Skin infection
Skin irritation
Skin lesion
Skin maceration
Skin mass
Skin necrosis
Skin oedema
Skin plaque
Skin reaction
Skin swelling
Skin tightness
Skin toxicity
Skin ulcer
Skin ulcer haemorrhage
Skin wound
Stevens-Johnson syndrome
Subacute cutaneous lupus erythematosus
Subcutaneous abscess
Subcutaneous haematoma

Swelling face
Swelling of eyelid
Swollen tongue
Systemic lupus erythematosus
Systemic lupus erythematosus rash
Systemic sclerosis
Thrombocytopenic purpura
Thrombotic thrombocytopenic purpura
Tongue oedema
Toxic epidermal necrolysis
Toxic skin eruption
Urticaria
Urticaria chronic
Urticaria papular
Vascular purpura
Vascular skin disorder
Vasculitic rash
Vasculitic ulcer
Weber-Christian disease
Xanthogranuloma
Xanthoma

13. Arthralgia (sponsor defined)

Arthralgia combines preferred term of arthralgia, pain in extremity, back pain, musculoskeletal pain, neck pain.

14. Arthralgia (steroid treated)

To identify the events of arthralgia that were treated with steroids (excluding topicals), the following steps are required.

- Identify the events of arthralgia (Arthralgia combines the preferred terms: of arthralgia, pain in extremity, back pain, musculoskeletal pain, neck pain), and Identify those arthralgia AEs that were treated with a concomitant medication after the onset date of the start of the arthralgia AE and before the resolution date of the arthralgia AE; and

- The resultant file will be clinically reviewed by BioMarin to identify the arthralgia events that were treated with a concomitant steroid (excluding those events that were administered topically, or where the steroid medication was used during an arthralgia episode but for an indication other than arthralgia).

15. Severe or Persistent (≥ 6 months) arthralgia

Arthralgia will be identified as the preferred terms of arthralgia, pain in extremity, back pain, musculoskeletal pain, and neck pain. Persistent arthralgia is defined as any arthralgia event that has a duration of the event ≥ 6 months (Multiple AE records with same reported term and ≤ 1 day difference between the start date and end date are considered as same event). Severe arthralgia is defined as any arthralgia event that is reported as CTCAE Grade 3 or above in severity.

16. Hypophenylalaninemia

Hypophenylalaninemia is defined as a blood phenylalanine (Phe) level ≤ 30 µmol/L for a minimum of 2 consecutive values, with the start of the event defined as the first blood Phe assessment day with < 30 µmol/L and the end of the event defined as the day prior to the first blood Phe assessment ≥ 30 µmol/L.

17: Neuropsychiatric disorder

A neuropsychiatric disorder is defined as Psychiatric disorder SOC + modified Nervous system SOC.

Name of Search	Preferred Term
Modified nervous SOC	Acalculia
	Agitation postoperative
	Agnosia
	Agraphia
	AIDS dementia complex
	Akathisia
	Altered state of consciousness
	Amnesia
	Amnestic disorder
	Anosognosia
	Anterograde amnesia
	Aphasia
	Apraxia

Aura
Autism
Borderline mental impairment
Circadian rhythm sleep disorder
Clumsiness
Cognitive disorder
Coprolalia
Crying
Decreased eye contact
Delayed sleep phase
Dementia
Depressed level of consciousness
Disorganised speech
Disorientation
Disturbance in attention
Dreamy state
Dysphemia
Dysphonia psychogenic
Dyssomnia
Echolalia
Fine motor skill dysfunction
Formication
Fumbling
Hyperkinesia
Hypersomnia
Hypokinesia
Hyporesponsive to stimuli
Hyposomnia
Incoherent
Initial insomnia
Insomnia
Intelligence increased
Irregular sleep phase
Judgement impaired
Lethargy
Memory impairment

Mental impairment
Mental retardation
Mild mental retardation
Moderate mental retardation
Mutism
Neurological decompensation
Neurological symptom
Parosmia
Phantom pain
Poor quality sleep
Posturing
Presenile dementia
Profound mental retardation
Psychomotor hyperactivity
Psychomotor skills impaired
Repetitive speech
Restlessness
Sedation
Senile dementia
Sleep phase rhythm disturbance
Slow response to stimuli
Slow speech
Somnolence
Speech disorder
Stereotypy
Stupor
Sudden onset of sleep
Symbolic dysfunction
Unresponsive to stimuli
Verbigeration

18: Hypersensitivity AEs

Hypersensitivity AEs will be identified in two ways:

- Broad Algorithmic anaphylactic reaction Standardized MedDRA Queries (SMQ) with the below algorithm:

  Algorithm = A or (B and C) or (D and (B or C))

- Modified Hypersensitivity SMQ to include below additional preferred terms

Name of Search	Preferred Term
Additional preferred terms for Modified Hypersensitivity SMQ	Arthralgia
	Arthritis
	Eye inflammation
	Eye irritation
	Eye pain
	Joint stiffness
	Joint swelling
	Pyrexia
	Vision blurred
	Polyarthritis

19: Arthralgia /Arthritis:

Arthralgia combines preferred term of arthralgia, Joint crepitation, Joint effusion, Joint destruction, Joint noise, Joint swelling, Joint stiffness.

Arthritis was identified using following preferred terms:

Name of Search	Preferred Term
Arthritis	Amyloid arthropathy
	Antithyroid arthritis syndrome
	Arthritis
	Arthritis allergic
	Arthritis climacteric
	Arthritis enteropathic
	Arthritis reactive
	Arthropathy
	Arthrotoxicity
	Autoimmune arthritis
	Behcet's syndrome
	Blau syndrome
	Carcinomatous polyarthritis
	Diabetic arthropathy
	Haemarthrosis
	Haemophilic arthropathy
	Hyper IgD syndrome
	Injection site joint inflammation
	Monarthritis
	Multicentric reticulohistiocytosis
	Neuropathic arthropathy
	Palindromic rheumatism
	Polyarthritis
	Poncet's disease
	Pyogenic sterile arthritis pyoderma gangrenosum and acne syndrome
	Reiter's syndrome
	Rheumatic fever
	Sacroiliitis
	Seronegative arthritis
	SLE arthritis
	Sympathetic posterior cervical syndrome
	Traumatic arthritis
	Traumatic arthropathy

20: Lymphadenopathy ≥ 14 Days Duration

Lymphadenopathy will be identified from preferred terms outlined below. To be included in the results, the duration of the event should be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Lymphadenopathy	Abdominal lymphadenopathy
	Hilar lymphadenopathy
	Injection site lymphadenopathy
	Lymph node calcification
	Lymph node fibrosis
	Lymph node pain
	Lymphadenitis
	Lymphadenocyst
	Lymphadenopathy
	Lymphadenopathy mediastinal
	Lymphatic disorder
	Lymphoid tissue hyperplasia
	Paratracheal lymphadenopathy
	Persistent generalised lymphadenopathy
	Retroperitoneal lymphadenopathy
	Spleen disorder
	Splenomegaly
	lymph node palpable

21: Persistent Urticaria ≥ 14 Days Duration

Persistent urticaria will be identified using preferred term: urticaria, with event duration ≥ 14 days (Multiple AE records with same reported term and ≤ 1day difference between the start date and end date and with severity worsens were considered as same event).

22: Infection and Infestations ≥ 30 Days Duration

Infection and Infestations will be identified using Infection and Infestations SOC. To be included in the result, the duration of the event should be ≥ 30 days (Multiple AE records with same reported term and ≤ 1day difference between the start date and end date and with severity worsens were considered as same event).

23: Ischemic Heart Disease

Ischemic heart disease will be identified using myocardial infarction narrow SMQ + Other Ischemic heart disease SMQ

24: Proteinuria/Albuminuria

Proteinuria/Albuminuria will be identified using the modified Proteinuria SMQ (narrowed SMQ adding a couple of the terms from the broad SMQ).

Name of Search	Preferred Term
Proteinuria/Albuminuria	Albumin globulin ratio increased
	Albumin urine present
	Albuminuria
	Bence Jones protein urine present
	Bence Jones proteinuria
	Beta 2 microglobulin urine increased
	Globulinuria
	Microalbuminuria
	Myoglobinuria
	Orthostatic proteinuria
	Protein urine
	Protein urine present
	Proteinuria
	Urine albumin/creatinine ratio abnormal
	Urine albumin/creatinine ratio increased
	Urine protein/creatinine ratio abnormal
	Urine protein/creatinine ratio increased
	Aminoaciduria
	Haemoglobinuria

25: Hematuria

Hematuria was identified using following preferred terms:

Name of Search	Preferred Term
Hematuria	Haematuria
	Haemoglobin urine present
	Haemoglobin urine
	Haemoglobinuria
	Red blood cells urine positive
	Red blood cells urine
	Blood urine present
	Blood urine

26: Blood Creatinine Increased

Blood creatinine increased will be identified using the following preferred terms: Blood creatinine abnormal, blood creatinine increased.

27: Renal Impairment

Renal impairment will be identified using a combination of modified narrow tubulointerstitial diseases SMQ, modified narrow acute renal failure SMQ, and modified narrow chronic kidney disease SMQ.

Name of Search	Preferred Term
Modified narrow tubulointerstitial diseases (SMQ)	Acidosis hyperchloraemic
	Acquired aminoaciduria
	Acute phosphate nephropathy
	Aminoaciduria
	Beta-N-acetyl-D-glucosaminidase increased
	Crystal nephropathy
	Eosinophils urine present
	Fanconi syndrome acquired
	Hyperphosphaturia
	Isosthenuria
	Nephritis allergic
	Nephrogenic diabetes insipidus
	Nephropathy toxic
	Renal glycosuria
	Renal papillary necrosis
	Renal tubular acidosis

	Renal tubular atrophy
	Renal tubular disorder
	Tubulointerstitial nephritis
	Tubulointerstitial nephritis and uveitis syndrome
	Urine phosphorus increased
	Urine retinol binding protein increased
	Albumin urine present
	Albuminuria
	Cylindruria
	Haematuria
	Haemoglobin urine present
	Haemoglobinuria
	Microalbuminuria
	Protein urine present
	Proteinuria
	Red blood cells urine positive
	Renal tubular necrosis
	Sterile pyuria

Name of Search	Preferred Term
Modified narrow acute renal failure (SMQ)	Acute kidney injury
	Acute phosphate nephropathy
	Acute prerenal failure
	Anuria
	Azotaemia
	Continuous haemodiafiltration
	Dialysis
	Haemodialysis
	Haemofiltration
	Neonatal anuria
	Nephropathy toxic
	Oliguria
	Peritoneal dialysis
	Prerenal failure
	Renal failure
	Renal failure neonatal

	Renal impairment
	Renal impairment neonatal
	Albuminuria
	Blood creatinine abnormal
	Blood creatinine increased
	Crystal nephropathy
	Hypercreatininaemia
	Nephritis
	Protein urine present
	Proteinuria
	Renal function test abnormal
	Urine output decreased

Name of Search	Preferred Term
Modified narrow chronic kidney disease (SMQ)	Artificial kidney device user
	Azotaemia
	Chronic kidney disease
	Coma uraemic
	Diabetic end stage renal disease
	Dialysis
	Dialysis device insertion
	Glomerulonephritis chronic
	Haemodialysis
	Haemofiltration
	Hepatorenal failure
	High turnover osteopathy
	Hyperparathyroidism secondary
	Kidney fibrosis
	Low turnover osteopathy
	Nephrogenic anaemia
	Nephrogenic systemic fibrosis
	Nephrosclerosis
	Oedema due to renal disease
	Pericarditis uraemic
	Peritoneal dialysis
	Renal and liver transplant

Renal and pancreas transplant
Renal failure
Renal osteodystrophy
Renal replacement therapy
Renal rickets
Renal transplant
Uraemia odour
Uraemic acidosis
Uraemic encephalopathy
Uraemic gastropathy
Uraemic neuropathy
Uraemic pruritus
Uridrosis
Albumin urine present
Albuminuria
Blood creatinine abnormal
Blood creatinine increased
Fibrillary glomerulonephritis
Focal segmental glomerulosclerosis
Glomerulonephritis
Glomerulonephritis membranoproliferative
Glomerulonephritis membranous
Glomerulonephritis minimal lesion
Glomerulonephritis proliferative
Glomerulonephritis rapidly progressive
Glomerulonephropathy
Glomerulosclerosis
Goodpasture's syndrome
IgA nephropathy
Immunotactoid glomerulonephritis
Mesangioproliferative glomerulonephritis
Microalbuminuria
Nephritic syndrome
Nephropathy
Nephropathy toxic
Nephrotic syndrome
Protein urine present

	Proteinuria
	Red blood cells urine positive
	Renal papillary necrosis
	Ultrafiltration failure
	Urinary casts present
	Urine albumin/creatinine ratio abnormal
	Urine albumin/creatinine ratio increased
	Urine output decreased
	Urine protein/creatinine ratio abnormal
	Urine protein/creatinine ratio increased

28: Increased liver function test or Events Involving Hepatic Impairment

Increased liver function test (LFT) or events involving hepatic impairment will be identified using modified hepatocellular damage and hepatitis NEC HLT plus broad Liver related investigations, signs and symptoms SMQ.

Additional preferred terms for modified hepatocellular damage and hepatitis NEC HLT:

Name of Search	Preferred Term
Additional preferred terms for modified hepatocellular damage and hepatitis NEC HLT	Autoimmune hepatitis
	Allergic hepatitis
	Chronic hepatitis
	Drug-induced liver injury
	Hepatitis
	Hepatitis acute
	Hepatitis fulminant
	Hepatitis toxic
	Hepatocellular injury
	Hepatotoxicity
	Liver injury
	Non-alcoholic fatty liver
	Non-alcoholic steatohepatitis
	Nonalcoholic fatty liver disease
	Steatohepatitis

Name of Search	Preferred Term
Liver related investigations, signs and symptoms (SMQ)	Alanine aminotransferase abnormal
	Alanine aminotransferase increased
	Ammonia abnormal
	Ammonia increased
	Ascites
	Aspartate aminotransferase abnormal
	Aspartate aminotransferase increased
	Bacterascites
	Bile output abnormal
	Bile output decreased
	Biliary ascites
	Bilirubin conjugated abnormal
	Bilirubin conjugated increased
	Biopsy liver abnormal
	Blood bilirubin abnormal
	Blood bilirubin increased
	Blood bilirubin unconjugated increased
	Bromosulphthalein test abnormal
	Child-Pugh-Turcotte score increased
	Computerised tomogram liver
	Foetor hepaticus
	Galactose elimination capacity test abnormal
	Galactose elimination capacity test decreased
	Gamma-glutamyltransferase abnormal
	Gamma-glutamyltransferase increased
	Guanase increased

Hepaplastin abnormal
Hepaplastin decreased
Hepatic artery flow decreased
Hepatic congestion
Hepatic enzyme abnormal
Hepatic enzyme decreased
Hepatic enzyme increased
Hepatic function abnormal
Hepatic hydrothorax
Hepatic hypertrophy
Hepatic mass
Hepatic pain
Hepatic sequestration
Hepatic vascular resistance increased
Hepatobiliary scan abnormal
Hepatomegaly
Hepatosplenomegaly
Hyperammonaemia
Hyperbilirubinaemia
Hypercholia
Hypertransaminasaemia
Kayser-Fleischer ring
Liver function test abnormal
Liver function test increased
Liver induration
Liver palpable
Liver scan abnormal
Liver tenderness
Mitochondrial aspartate aminotransferase increased

Molar ratio of total branched-chain amino acid to tyrosine
Oedema due to hepatic disease
Perihepatic discomfort
Retrograde portal vein flow
Total bile acids increased
Transaminases abnormal
Transaminases increased
Ultrasound liver abnormal
Urine bilirubin increased
X-ray hepatobiliary abnormal
5'nucleotidase increased
Blood alkaline phosphatase abnormal
Blood alkaline phosphatase increased
Blood cholinesterase abnormal
Blood cholinesterase decreased
Deficiency of bile secretion
Glutamate dehydrogenase increased
Haemorrhagic ascites
Hepatic fibrosis marker abnormal
Hepatic fibrosis marker increased
Hypoalbuminaemia
Leucine aminopeptidase increased
Liver iron concentration abnormal
Liver iron concentration increased
Periportal oedema
Peritoneal fluid protein abnormal
Peritoneal fluid protein decreased
Peritoneal fluid protein increased
Pneumobilia

	Portal vein flow decreased
	Portal vein pressure increased
	Retinol binding protein decreased
	Urobilinogen urine decreased
	Urobilinogen urine increased

29: Anemia

Anemia will be identified using following preferred terms:

Name of Search	Preferred Term
Anemia	Anaemia
	Anaemia macrocytic
	Anaemia megloblastic
	Hyperchromic anaemia
	Hypochromic anaemia
	Microcytic anaemia
	Sideroblastic anaemia

30: Thrombocytopenia

Thrombocytopenia will be identified using following preferred terms:

Name of Search	Preferred Term
Thrombocytopenia	Thrombocytopenia
	Immune thrombocytopenic purpura
	Platelet production decreased
	Thrombocytopenic purpura
	Platelet count decreased
	Platelet count abnormal
	Platelet destruction increased
	Platelet disorder
	Platecrit abnormal
	Platecrit decreased

31: Hemolysis

Hemolysis will be identified using broad haemolytic disorders SMQ.

Name of Search	Preferred Term
Hemolysis	ABO haemolytic disease of newborn
	ABO incompatibility

	Acid haemolysin test positive
	Acute haemolytic transfusion reaction
	Alloimmunisation
	Anti A antibody positive
	Anti B antibody positive
	Anti-erythrocyte antibody positive
	Autoimmune haemolytic anaemia
	Blood incompatibility haemolytic anaemia of newborn
	Cardiac haemolytic anaemia
	Cold agglutinins positive
	Cold type haemolytic anaemia
	Coombs direct test positive
	Coombs indirect test positive
	Coombs negative haemolytic anaemia
	Coombs positive haemolytic anaemia
	Coombs test positive
	Delayed haemolytic transfusion reaction
	Erythroblastosis
	Erythroblastosis foetalis
	Evans syndrome
	Extravascular haemolysis
	Glucose-6-phosphate dehydrogenase abnormal
	Glucose-6-phosphate dehydrogenase deficiency
	Haemoglobin urine present
	Haemoglobinaemia
	Haemoglobinuria
	Haemolysis
	Haemolysis neonatal
	Haemolytic anaemia
	Haemolytic transfusion reaction
	Haemolytic uraemic syndrome
	Haemosiderinuria
	Haptoglobin decreased
	Intravascular haemolysis
	Isoimmune haemolytic disease
	Jaundice acholuric
	Microangiopathic haemolytic anaemia

	Paroxysmal nocturnal haemoglobinuria
	Passenger lymphocyte syndrome
	Red cell fragmentation syndrome
	Rhesus antibodies positive
	Rhesus haemolytic disease of newborn
	Rhesus incompatibility
	Transfusion reaction
	Warm type haemolytic anaemia
	Reticulocyte count abnormal
	Reticulocyte count increased
	Reticulocyte percentage abnormal
	Reticulocyte percentage increased
	Reticulocytosis

32: Myalgia

Myalgia will be identified using the following PT: Fibromyalgia, Myalgia, Myalgia intercostal, Myofascial pain syndrome, Neuromuscular pain

33: Serositis

Serositis will be identified using following preferred terms:

Name of Search	Preferred Term
Serositis	Pericardial effusion
	Pericardial rub
	Pericarditis
	Pleural effusion
	Pleural rub
	Pleurisy
	Pleuropericarditis
	Polyserositis
	Serositis
	Peritonitis
	Noninfectious peritonitis
	Ascites
	Lymphocele
	Chylothorax

34: Potential central nervous system vascular complications

Potential central nervous system (CNS) vascular complications will be identified using ischaemic central nervous system vascular conditions (SMQ).

105 Digital Drive

Novato, CA 94949

Statistical Analysis Plan

Study 165-306

Version 2.0

5 March 2025

1 APPROVALS (SIGNATURE AND DATE)

Title: A Phase 3 Multi-Center Study to Evaluate the Safety and Efficacy of Subcutaneous Injections of Pegvaliase in Adolescent Subjects (Ages 12-17) with Phenylketonuria Featuring an Open-Label Randomized Two-Arm (Active vs Diet-Only Control) Design

Protocol: 165-306 Amendment 2, 05Mar2025

Date: March 5, 2025

{Please See Appended Electronic Signature Page}

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2 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALT	alanine transaminase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
C3	complement component 3
C4	complement component 4
CRP	C-reactive protein
CRF	Case Report Form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DBP	diastolic blood pressure
ECG	electrocardiogram
HAE	hypersensitivity adverse event
IgE	immunoglobulin E
IgG	immunoglobulin G
IgM	immunoglobulin M
ITT	intent-to-treat
IWRS	Interactive Web Response System
LOCF	last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
Nab	neutralizing antibody
NIAID/FAAN	National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network
PAL	Phenylalanine ammonia-lyase
PEG	polyethylene glycol
Phe	phenylalanine
PK	pharmacokinetics
PKU	phenylketonuria
PT	preferred term

SAP	statistical analysis plan
SBP	systolic blood pressure
SD	standard deviation
SOC	system organ class
SMQ	Standardized MedDRA Queries
TEAE	treatment-emergent adverse event
Tab	total antibody
WHO Drug	World Health Organization Drug Dictionary

3 INTRODUCTION

Study 165-306 is an open-label, two-arm (Active vs Diet-only Control), randomized control study to evaluate safety and efficacy of pegvaliase and characterize the PK of pegvaliase in adolescent participants with phenylketonuria (PKU).

The purpose of this Statistical Analysis Plan (SAP) is to provide a comprehensive description of methods of the data analyses outlined in the protocol (amendment 2 dated 23 October 2023).

If discrepancies exist between the text of the statistical analysis as planned in the protocol and the final SAP, the SAP will prevail. The discrepancies will be documented in the clinical study report (CSR).

3.1 Objectives of Study

The primary objective of the study is:

- To evaluate the efficacy of pegvaliase in adolescent participants with PKU
- To evaluate the safety of pegvaliase in adolescent participants with PKU

The secondary objectives of the study are:

- To characterize total dietary protein intake in adolescent participants with PKU after pegvaliase treatment
- To characterize the pharmacokinetics (PK) of pegvaliase in adolescent participants with PKU

The exploratory objectives of the study are:

- To evaluate the effect of pegvaliase treatment on neurocognitive outcomes in adolescent participants with PKU
- To assess changes in PKU symptoms and impacts
- To compare participants' optimal maintenance pegvaliase dose across different age groups within the study
- Explore the biochemical, molecular, and cellular aspects of PKU
- PAH genotyping

3.2 Study Design

This is an open-label, two arm, randomized controlled study. Approximately 54 US and EU participants (ages 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive) with diet-only control will be enrolled. At enrollment, participants will be randomized 2:1 to the active (pegvaliase) and control (diet-only) treatment arms, with approximately 36 participants receiving daily pegvaliase and 18 participants managing PKU by diet alone. Treatment assignment will be stratified by Screening/Run-in blood Phe (mean of the 2 pre-

treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L. Participants in the active and control arms will follow the same visit schedules and perform the same assessments throughout the 72-week Primary Treatment Phase (Part 1) except that the control participants will not be receiving pegvaliase so will not have PK draws or immunogenicity assessments.

After providing informed consent, participants will enter the Screening/Run-in period, which will last 4 weeks and include 2 assessments of blood Phe levels, 7 to 10 days apart, to determine eligibility. Following confirmation that the 2 blood Phe results are > 600 µmol/L and all other eligibility criteria have been met, participants may be enrolled in the study on Day 1. During the Screening/Run-in and the Primary Treatment Phase (Part 1), participants will be required to maintain stable protein intake from both medical food and/or intact food.

The duration of Part 1 is 72 weeks, during which pegvaliase treatment will be initiated for participants in the active arm using an Induction/Titration/Maintenance (I/T/M) dosing regimen. The I/T/M dosing regimen consists of an Induction/Titration Dosing (9 weeks) and a Maintenance Dosing. Required hospital setting visits during Part 1 will occur for all dosing visits for the first 8 weeks, every 4 weeks thereafter, and when the pegvaliase dose is escalated to 20, 40 or 60 mg/day. The assessments performed at the clinic visits include measurement of blood Phe, neurocognitive assessments, PK blood draws (active participants only), and safety assessments.

Beginning Week 73, participants in the active treatment arm will continue to receive pegvaliase in the Extension Phase (Part 2) for up to 142 additional weeks (through Week 215). Beginning Week 73, participants in the control treatment arm will initiate pegvaliase treatment and, from Weeks 73 through 215, will follow the same schedule for dosing, study visits, and assessments that the active arm participants followed for pegvaliase dosing starting from Study Day 1.

3.3 Study Population

Individuals with PKU aged 12 to 17 years old (US), inclusive, and 12 to 15 years old (EU), inclusive with diet-only control at the start of the Screening/Run-in Period (Day -28) are candidates for participation in the study. Additional criteria for study participation are presented in Protocol 165-306 Section 5.1 and Section 5.2.

3.4 Study Dosage and Administration

Participants will receive pegvaliase at a concentration of 5.0 or 20.0 mg/mL. Pegvaliase will be administered subcutaneously at dose levels in the range of 2.5 to 60 mg. The minimum

dose of pegvaliase is 2.5 mg/week. The maximum allowable daily dose of pegvaliase is 60 mg/day (for a maximum weekly total dose of 420 mg).

3.5 Sample Size Determination

Approximately 54 participants will be enrolled and randomized to the pegvaliase or diet-only groups in 2:1 ratio. Assuming a mean (SD) reduction in Phe following 72 weeks on study is 640 (570) µmol/L in the pegvaliase group and 100 (250) µmol/L in the diet-only group, a total sample size of 54 participants will provide more than 90% power to detect a treatment difference, based on the two-sample T-test with unequal variance and a significance level of 0.05 (two sided).

Approximately 72 participants will be screened for study participation such that approximately 54 participants will be enrolled in the study.

For the pegvaliase treatment arm, the mean (SD) assumption of reduction in blood Phe is based on participants 16 to 24 years of age who completed 17 months of treatment in previous pegvaliase studies utilizing an I/T/M dosing regimen (n = 46). For the diet-only arm, the mean (SD) assumption of reduction in blood Phe is based on BioMarin's PKUDOS (Phenylketonuria Demographic, Outcomes, and Safety) registry for patients aged 12 to <18 years old on diet only control with baseline Phe > 600 µmol/L at year 1 of study enrollment (n = 57).

3.6 Blinding and Randomization Methods

3.6.1 Blinding Method

Participants will receive open-label pegvaliase or diet-only treatment.

3.6.2 Randomization Method

Participants will be randomized in a 2:1 ratio to the active (pegvaliase) or control (diet-only) treatment arm, respectively. Treatment assignment will be stratified by Screening/Run-In blood Phe (mean of the 2 pre-treatment blood Phe assessments performed during the Screening/Run-in period) of ≤ 1000 µmol/L or > 1000 µmol/L.

3.7 Interim Analysis

There are no interim analyses planned. The primary analysis will be performed after all participants complete Part 1 or discontinued from the study. The final analyses will be performed after all participants have completed the study or discontinued from the study.

4 GENERAL ANALYSIS CONSIDERATION

Descriptive summaries of continuous variables will include n, mean, standard deviation (SD), median, minimum, and maximum. Descriptive summaries of categorical variables will include n, frequency, and percentage.

4.1 Analysis Populations

The All Enrolled population will consist of all participants who agree to participate in the clinical study (by their parent/guardians, if applicable) following completion of the informed consent process and determination of study eligibility.

The Intent to Treat (ITT) population will consist of all participants who are randomized. Participants will be analyzed according to the randomized treatment for the analysis of efficacy.

The Safety population will consist of all participants who received at least one dose of study treatment (pegvaliase or diet-only management of PKU). Subjects will be analyzed according to the treatment actually received for the analysis of safety.

The Per Protocol (PP) population will consist of all participants in the ITT population who are compliant with the protocol with no important protocol deviations impacting the primary efficacy analysis. The PP population will be determined by the study team through data review before database snapshot for primary analysis; reasons for excluding participants will be defined and documented prior to database snapshot for primary analysis.

The PK population will consist of all participants who received pegvaliase with at least one PK measurement.

4.2 Treatment Group Presentation

The following treatment groups will be used in summary tables:

Part 1: pegvaliase (active) or diet-only (control)

Part 2: pegvaliase (participants continuing on pegvaliase) or diet-only (participants transitioning from control to pegvaliase)

4.3 Pooling of Data from Sites with Small Enrollment

Since the enrollment for each site is expected to be small, the analyses will not be performed by site or pooled sites.

4.4 Study Day Derivation

Study days will be derived as follows:

Pegvaliase arm: the first dose date will be assigned as Study Day 1

Diet only arm: the Visit 1 date will be assigned as Study Day 1

For visit occurs on or after Study Day 1, Study Day = visit date – day 1 date + 1

For visit occurs before Study Day 1, Study Day = visit date – day 1 date. There is no Study Day 0.

4.4.1 Baseline Definition

The baseline for all safety and efficacy endpoints (when applicable) is defined as the last non-missing measurement collected prior to the first pegvaliase dose (pegvaliase arm) or prior to or on Visit 1 date (diet-only arm), unless otherwise specified:

Baseline blood Phe concentration at Part 1 is defined as the average of 3 pre-treatment measurements collected between screening/Run-in (2) and Day 1 pre-dose (1). Baseline blood Phe concentration at Part 2 is defined as the average of week 69 and week 73 measurements.

Baseline total protein intake at Part 1 is defined as the mean total dietary protein intake calculated from the 3 separate 3-day diet diaries collected during Screening/Run-in (2) and Day 1 (1). Baseline total protein intake at Part 2 is defined as the average of week 69 and week 73 measurements

For other measurements,

- Pegvaliase:
    - Baseline for the whole study period: last non-missing measurement prior to first pegvaliase dose
- Diet only:
    - Baseline for Part 1: last non-missing measurement prior to visit day 1
    - Baseline for Part 2: measurements taken prior to first dose of pegvaliase at Week 73

4.4.2 Treatment Period and Phase

Pegvaliase treatment period is defined from study Day 1 until 30 days after last dose.

Definitions of Induction, titration and maintenance phase during pegvaliase treatment period are as follows:

Induction: When subjects are on a 2.5 mg weekly dose.

Titration: When subjects have completed the induction phase and begin to increase dose and dosing frequency until they meet the criterion for the Maintenance Phase.

Induction/Titration: The treatment period prior to the start of Maintenance Phase.

Maintenance (first occurrence):

- US: Completion of an 8-week period with at least 80% compliance at the same dose. The start of maintenance phase is at the end of the 8-week period.
- EU: when subjects have at least a 26-day period with all blood Phe assessments $\leq$ 600 µmol/L and are on stable dose, which is defined as on same dose with at least 80% compliance rate. The start of maintenance phase is the date of the first Phe assessment at which the above criteria are met.

Diet-only period is defined from study Day 1 until the day before first dose of pegvaliase or early termination or data cut-off date, whichever occurs earlier.

4.5 Visit Window for Analysis

All efficacy and safety data will be summarized by study week of assessment. An assessment for a subject will be classified according to the study day of the assessment where it falls within a window. The windows are designated for each scheduled week of visit and centered on a target day. If there are two or more assessments within a designated window, the assessment that is closest to the target day will be used for analyses. If the two closest assessments to the target day are equidistant from the target day, then for numerical variable, the average of the two assessments will be used for analyses. For categorical variables, the worst of the 2 equidistant measurements, indicating less treatment effect, will be used.

Table 4.5.1 lists the scheduled weeks of the assessments collected in this study and the corresponding range of treatment days (window) during which a visit may have occurred.

Table 4.5.1: Visit Time Windows for the Treatment Period

Assessment: Blood Phe, Tyrosine, 3-day diet diary

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 63
	Week XX (11 ≤ XX ≤ 19, every two weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 6 to Day (XX–1)*7 + 7
	Week 21	Day 141	Day 134 to Day 154
	Week XX (25≤XX≤73, every four weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14
Part 2	Week XX (73<XX≤209, every four weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14
	Week 213	Day 1485	Day 1471 to Day 1491
	Week 215	Day 1499	≥ Day 1492

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 - 2 to Day (XX – 1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤ XX ≤ 69, every four weeks)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 -13 to Day (XX -1)*7 + 14
	Week 73	Day 505	Day 491 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)		Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3

		Week 73 day + (XX – 73)*7	
	Week 81	Week 73 day + 56	Week 73 day + 53 to Week 73 day + 62
	Week XX (83 ≤ XX ≤ 91, every two weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 7 to Week 73 day + (XX - 73)*7 + 6
	Week 93	Week 73 day + 140	Week 73 day + 133 to Week 73 day + 153
	Week XX (97≤XX ≤ 209, every four weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 14 to Week 73 day + (XX - 73)*7 + 13
	Week 213	Day 1485	Week 73 day + 966 to Week 73 day + 986
	Week 215	Day 1499	≥ Week 73 day + 987

Assessment: Physical examination, weight, vital sign, clinical safety laboratory test

Pegvaliase	**Derived Visit**	**Target Day[a]**	**Window[b]**
	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤XX≤209, every 4 weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX – 1)*7 + 14
	Week 213	Day 1485	Day 1471 to Day 1491
	Week 215	Day 1499	≥ Day 1492

Diet-Only	**Derived Visit**	**Target Day[a]**	**Window[b]**
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 - 2 to Day (XX – 1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤ XX ≤ 69, every 4 weeks)	Day (XX – 1) *7 + 1	Day (XX - 1)*7 -13 to Day (XX -1)*7 + 14
	Week 73	Day 505	Day 491 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3
	Week 81	Week 73 day + 56	Week 73 day + 53 to Week 73 day + 69
	Week XX (85 ≤ XX ≤ 209, every 4 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 14 to Week 73 day + (XX - 73)*7 + 13
	Week 213	Week 73 day + 980	Week 73 day + 966 to Week 73 day + 986
	Week 215	Week 73 day + 994	≥ Week 73 day + 987

Assessment: C-Reactive protein, urine albumin/creatinine ratio

Pegvaliase	**Derived Visit**	**Target Day[a]**	**Window[b]**
	Screening/Day 1	Day 1	≤ Day 1
	Week 9	Day 57	Day 2 to Day 84
	Week XX (17 ≤ XX≤ 201, every 8 weeks)	Day (XX – 1)*7 + 1	Day (XX - 3)*7 - 13 to Day (XX +1)*7 + 14
	Week 209	Day 1457	Day 1429 to Day 1477
	Week 215	Day 1499	≥ Day 1478

Diet-Only	**Derived Visit**	**Target Day[a]**	**Window[b]**
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 9	Day 57	Day 2 to Day 84
	Week XX (17 ≤ XX ≤ 65, every 8 weeks)	Day (XX – 1) *7 + 1	Day (XX - 3)*7 - 13 to Day (XX + 1)*7 + 14
	Week 73	Day 505	Day 477 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 81	Week 73 day + 56	Week 73 day + 1 to Week 73 day + 83
	Week XX (89 ≤ XX ≤ 201, every 8 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 – 28 to Week 73 day + (XX - 73)*7 + 27

	Week 209	Week 73 day + 952	Week 73 day + 924 to Week 73 day + 972
	Week 215	Week 73 day + 994	≥ Week 73 day + 973

Assessment: Complements C3 and C4

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 5	Day 29	Day 2 to Day 42
	Week XX (9 ≤ XX≤ 21, every 4 weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX -1)*7 + 14
	Week 25	Day 169	Day 155 to Day 196
	Week XX (33 ≤ XX≤ 177, every 8 weeks)	Day (XX – 1)*7 + 1	Day (XX - 3)*7 - 13 to Day (XX +1)*7 + 14
	Week 185	Day 1289	Day 1261 to Day 1302
	Week 189	Day 1317	Day 1303 to Day 1330
	Week 193	Day 1345	Day 1331 to Day 1372
	Week 201	Day 1401	Day 1373 to Day 1428
	Week 209	Day 1457	Day 1429 to Day 1477
	Week 215	Day 1499	≥ Day 1478

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 5	Day 29	Day 2 to Day 42
	Week 9	Day 57	Day 43 to Day 84
	Week 17	Day 113	Day 85 to Day 140
	Week 25	Day 169	Day 141 to Day 196
	Week XX (33 ≤ XX ≤ 65, every 8 weeks)	Day (XX – 1) *7 + 1	Day (XX - 3)*7 -13 to Day (XX +1)*7 + 14
	Week 73	Day 505	Day 477 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 77	Week 73 day + 28	Week 73 day + 1 to Week 73 day + 41
	Week XX (81 ≤ XX ≤ 93, every 4 weeks)		Week 73 day + (XX - 73)*7 – 14 to Week 73 day + (XX - 73)*7 +13

		Week 73 day + (XX – 73)*7	
	Week 97	Week 73 day + 168	Week 73 day + 154 to Week 73 day + 195
	Week XX (105 ≤ XX ≤ 201, every 8 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 – 28 to Week 73 day + (XX - 73)*7 + 27
	Week 209	Week 73 day + 952	Week 73 day + 924 to Week 73 day + 972
	Week 215	Week 73 day + 994	≥ Week 73 day + 973

Assessment: PK

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 5	Day 29	Day 2 to Day 42
	Week XX (9 ≤ XX≤ 21, every 4 weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX -1)*7 + 14
	Week 25	Day 169	Day 155 to Day 196
	Week XX (33 ≤ XX≤ 201, every 8 weeks)	Day (XX – 1)*7 + 1	Day (XX - 3)*7 - 13 to Day (XX +1)*7 + 14
	Week 209	Day 1457	Day 1429 to Day 1477
	Week 215	Day 1499	≥ Day 1478

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 77	Week 73 day + 28	Week 73 day + 1 to Week 73 day + 41
	Week XX (81 ≤ XX ≤ 93, every 4 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 – 14 to Week 73 day + (XX - 73)*7 +13
	Week 97	Week 73 day + 168	Week 73 day + 154 to Week 73 day + 195
	Week XX (105 ≤ XX ≤ 201, every 8 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 – 28 to Week 73 day + (XX - 73)*7 + 27
	Week 209	Week 73 day + 952	Week 73 day + 924 to Week 73 day + 972
	Week 215	Week 73 day + 994	≥ Week 73 day + 973

Assessment: Immunogenicity assessment

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 2	Day 8	Day 2 to Day 11
	Week XX (3 ≤ XX ≤ 8, every week)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 2 to Day (XX–1)*7 + 4
	Week 9	Day 57	Day 54 to Day 70
	Week XX (13 ≤ XX ≤ 21, every 4 weeks)	Day (XX – 1)*7 + 1	Day (XX - 1)*7 - 13 to Day (XX–1)*7 + 14
	Week 25	Day 169	Day 155 to Day 196
	Week XX (33≤XX≤209, every 8 weeks)	Day (XX – 1)*7 + 1	Day (XX - 3)*7 - 13 to Day (XX + 1)*7 + 14
	Week 209	Day 1457	Day 1429 to Day 1477
	Week 215	Day 1499	≥ Day 1478

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 74	Week 73 day + 7	Week 73 day + 1 to Week 73 day + 10
	Week XX (75 ≤ XX ≤ 80, every week)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 3 to Week 73 day + (XX - 73)*7 + 3
	Week 81	Week 73 day + 56	Week 73 day + 53 to Week 73 day + 69
	Week 85	Week 73 day + 84	Week 73 day + 70 to Week 73 day + 97
	Week 89	Week 73 day + 112	Week 73 day + 98 to Week 73 day + 125
	Week 93	Week 73 day + 140	Week 73 day + 126 to Week 73 day + 153
	Week 97	Week 73 day + 168	Week 73 day + 154 to Week 73 day + 195
	Week XX (105 ≤ XX ≤ 201, every 8 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 – 28 to Week 73 day + (XX - 73)*7 + 27
	Week 209	Week 73 day + 952	Week 73 day + 924 to Week 73 day + 972
	Week 215	Week 73 day + 994	≥ Week 73 day + 973

Assessment: ADHD, BRIEF2, PKU-SSIS, PGIS, CaGIS, CGIS

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 13	Day 85	Day 2 to Day 126
	Week XX (25 ≤ XX ≤ 205, every 12 weeks)	Day (XX – 1)*7 + 1	Day (XX - 5)*7 - 13 to Day (XX+3)*7 + 14
	Week 205	Day 1429	Day 1387 to Day 1463
	Week 215	Day 1499	≥ Day 1464

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 13	Day 85	Day 2 to Day 126
	Week XX (25 ≤ XX ≤ 61, every 12 weeks)	Day (XX – 1) *7 + 1	Day (XX - 5)*7 - 13 to Day (XX + 3)*7 + 14
	Week 73	Day 505	Day 463 to Day of first dose of pegvaliase
Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 85	Week 73 day + 84	Week 73 day + 1 to Week 73 day + 125
	Week XX (97 ≤ XX ≤ 193, every 12 weeks)	Week 73 day + (XX – 73)*7	Week 73 day + (XX - 73)*7 - 42 to Week 73 day + (XX - 73)*7 + 41
	Week 205	Week 73 day + 924	Week 73 day + 882 to Week 73 day + 958
	Week 215	Week 73 day + 994	≥ Week 73 day + 959

Assessment: PGIC, CaGIC

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 37	Day 253	Day 2 to Day 378
	Week XX (73 ≤ XX ≤ 145, every 36 weeks)	Day (XX – 1)*7 + 1	Day (XX - 17)*7 - 13 to Day (XX+15)*7 + 14
	Week 181	Day 1261	Day 1135 to Day 1379
	Week 215	Day 1499	≥ Day 1380

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 37	Day 253	Day 2 to Day 253
	Week 73	Day 505	Day 379 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 109	Week 73 day + 252	Week 73 day + 1 to Week 73 day + 377
	Week 145	Week 73 day + 506	Week 73 day + 378 to Week 73 day + 629
	Week 181	Week 73 day + 756	Week 73 day + 630 to Week 73 day + 874
	Week 215	Week 73 day + 994	≥ Week 73 day + 875

Assessment: Serum Cortisol

Pegvaliase	Derived Visit	Target Day[a]	Window[b]
	Screening/Day 1	Day 1	≤ Day 1
	Week 25	Day 169	Day 2 to Day 252
	Week 49	Day 337	Day 253 to Day 420
	Week 73	Day 505	Day 421 to Day 588
	Week 97	Day 673	Day 589 to Day 756
	Week 121	Day 841	Day 757 to Day 952
	Week 153	Day 1065	Day 953 to Day 1190
	Week 189	Day 1317	Day 1191 to Day 1442

Diet-Only	Derived Visit	Target Day[a]	Window[b]
Part 1	Screening/Day 1	Day 1	≤ Day 1
	Week 25	Day 8	Day 2 to Day 252
	Week 49	Day 57	Day 253 to Day 420
	Week 73	Day 505	Day 421 to Day of first dose of pegvaliase

Part 2	Week 73	Day of first dose of pegvaliase	Day of first dose of pegvaliase
	Week 97	Week 73 day + 168	Week 73 day + 1 to Week 73 day + 251
	Week 121	Week 73 day + 336	Week 73 day + 252 to Week 73 day + 419
	Week 145	Week 73 day + 504	Week 73 day + 420 to Week 73 day + 587
	Week 169	Week 73 day + 672	Week 73 day + 588 to Week 73 day + 755
	Week 193	Week 73 day + 840	Week 73 day + 756 to Week 73 day + 916
	Week 215	Week 73 day + 994	≥ Week 73 day + 917

[a] Target days are relative to the Study Day 1.
[b] Visit day is calculated by (visit date – Study Day 1 + 1).
Diet only group: all Part 2 visit is relative to the first dose day of pegvaliase

4.6 Handling of Dropouts and Missing Data

Participants who discontinued prematurely will not be replaced.

Missing dates or partially missing dates will be imputed conservatively for concomitant medications and AEs to ensure that an AE is considered treatment emergent when possible and has the longest possible duration. If the onset date of an AE is completely missing, the AE will be considered treatment-emergent. Missing data in blood Phe will be handled by multiple imputation method, more details are provided in Section 13.1.1

5 SUBJECT DISPOSITION

The total number of enrolled participants, the number (%) of participants who received study treatment, the number (%) of participants completed Part 1, the number (%) of participants who completed the study, and the number of participants in each analysis population (ITT, Safety, PP and PK), as appropriate, will be summarized.

6 DISCONTINUATION AND COMPLETION

For participants who prematurely discontinue the study, the primary reason for discontinuation will be summarized by treatment group. A similar summary will be provided for participants who discontinue study treatment. A subject listing of completion and early termination from study will also be provided.

7 PROTOCOL DEVIATIONS

Both major and minor protocol deviations will be summarized by deviation category. All protocol deviations will be listed. Participants with inclusion or exclusion criteria deviations will also be provided in a separate listing.

8 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Subject demographic and baseline characteristics will be summarized for the ITT population. Demographics include age, age category (12 to < 16 and 16 to < 18 years old), gender, race, country and ethnicity. Baseline characteristics include height, weight, BMI, blood Phe concentration, neurocognitive assessment, three-day average dietary intake, restricted diet, genotyping and antibody status.

9 MEDICAL HISTORY

Each subject's chronic diseases, disorders, and surgeries in the past will be collected as general medical history. General medical history will be summarized by System Organ Class (SOC) and Preferred Term (PT) by descending order of frequency. A by-subject listing of each subject's medical history will also be provided.

10 PRIOR AND CONCOMITANT MEDICATIONS/PROCEDURES

The following definitions will be used to determine prior and concomitant medications:

- Prior medications: any medications taken prior to first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Concomitant medications: any medications initially taken on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm).
- Prior and concomitant medications: any medications taken both prior to and on or after first dose administration of pegvaliase (pegvaliase arm) or study day 1 (diet-only arm) will be reported both as prior and concomitant medications. Therefore, any such medications where the stop date is reported, reported as "continuing", or missing will be considered as both prior and concomitant medications.

In the event the start date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the start date will be imputed as the first day of the month, if the year and month is same or after the study day 1 year and month. If only day is missing, then the start date will be imputed as the last day of the month, if the year and month is prior to the first dose year and month. If year and month are the same as

the year and month of study day 1 then the start date will be imputed as the date of study day 1.

- If only year is non-missing, then the start date will be imputed as the first day of the year if the year is after the year of study day 1. If year is the same as the year of study day 1 then the start date will be imputed as the date of study day 1. If year is before the year of study day 1 then start date will be imputed to the last date of the year.

In the event the stop date of a medication is partial, the following imputation rules will be applied:

- If only day is missing, then the end date will be imputed as the last day of the month.
- If only year is non-missing, then the end date will be imputed as the last day of the year.

All medications will be coded using the current version of the World Health Organization Drug (WHO Drug) dictionary (September 2021). Prior and concomitant medication use will be separately summarized by Anatomical Therapeutic Chemical (ATC) medication class (Level 4) and preferred name (i.e., generic medication name). A subject reporting the same medication use more than once will be counted once when calculating the number and percentage of participants who received that medication. A subject listing of each subject's prior and concomitant medications will be provided.

11 STUDY DRUG USAGE

Study drug usage rate will be calculated based on the drug exposure records captured in eCRFs and dispensed drug amount captured in the Interactive web response system (IWRS).

The overall study drug usage rate will be derived from the total amount of study drug intake divided by the total dispensed study drug amount over the study period and multiplied by 100%. The rate will be calculated for Part 1, Part 2 and overall study period.

12 EXTENT OF EXPOSURE TO STUDY TREATMENT

For pegvaliase treatment period, the total number of days on study treatment, total amount of study drug (mg) taken and average daily dose (mg) will be summarized for Part 1, Part 2 and overall study period. Any dose interruption longer than 28 days will be excluded from the total exposure duration calculation.

In addition, the total number of days on diet control treatment period will be summarized for diet-only arm, the summary of protein intake is described in Section 13.2.1

A by-subject listing of each subject's extent of exposure and compliance will be provided.

Compliance for pegvaliase will be calculated by the total amount of study drug intake divided by the total amount of study drug dispensed, and multiplied by 100%

13 EFFICACY EVALUATIONS

Unless otherwise specified, efficacy analysis will be based on the ITT population.

13.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in blood Phe following 72 weeks on study. The blood Phe measurement following 72 weeks on study is defined as the average of the Week 69 and Week 73 blood Phe measurements. Change from baseline in blood Phe will be compared between participants in the active (pegvaliase) and the control (diet-only) treatment arms.

13.1.1 Primary Estimand

Population: the target study population comprises adolescent participants (ages 12-17) with PKU who meet the inclusion and exclusion criteria as specified in the protocol. The analysis population is the ITT population as defined in Section 4.1

Variable: the variable is the primary efficacy endpoint, change from baseline in blood Phe following 72 weeks on study, as defined in Section 13.1.

Handling of missing data and intercurrent events (ICEs): the treatment policy strategy will be adopted which will include all blood Phe data regardless of intercurrent events such as treatment discontinuation. Missing blood Phe data will be imputed by multiple imputation methods as described in Section 13.1.2.

Population level summary: the difference in change from baseline in blood Phe following 72 weeks on study will be estimated by an Analysis of Covariance (ANCOVA) model with multiple imputation of missing data, as described in Section 13.1.2.

The hypotheses for the primary endpoint are:

H_0: Mean reduction in blood Phe of pegvaliase arm $\leq$ mean reduction in blood Phe of Diet-only arm

H_a: Mean reduction in blood Phe of pegvaliase arm > mean reduction in blood Phe of Diet-only arm

The significance level of the hypothesis testing is 0.05 two-sided (0.025 one-sided). The success criteria is pegvaliase arm demonstrates significantly greater reduction in blood Phe compared to Diet-only arm

13.1.2 Primary Analysis

The primary efficacy endpoint will be evaluated using an ANCOVA model, which will include baseline blood Phe levels as covariates, randomization strata (baseline Phe $\leq$ 1000 µmol/L or > 1000 µmol/L) and treatment group as factors, with missing data handled by multiple imputation. The imputation will be performed using the SAS procedure PROC MI; the variable list for imputation will include baseline and post-baseline blood Phe measurements and stratified by treatment. The MCMC method will be used to generate 100 imputation datasets. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. The primary analysis will be based on the ITT population.

Descriptive summary of observed values, change and percent change from baseline in blood Phe will be provided by visit and treatment

In Part 2, change and percent change in blood Phe from average of Week 69 and Week 73 visits will also be summarized for participants transitioning from control to pegvaliase by visits scheduled in Part 2.

13.1.3 Sensitivity Analysis

The same statistical model used in the primary analysis will be repeated in the PP population.

In addition, the primary analysis model (ANCOVA) will be repeated on the ITT population using 1) observed data only and 2) observed data and missing data imputed by Last Observation Carried Forward (LOCF).

13.2 Secondary Endpoints

The secondary endpoints are as follows:

- Change from baseline in total dietary protein intake (i.e., medical food and/or intact food) following 72 weeks on study. The total dietary protein intake following 72 weeks on study is defined as the mean total dietary protein intake calculated from the diet diaries collected at the Week 69 and Week 73 visits. Total dietary protein is measured in grams/kilogram (g/kg).
- Plasma sample for trough PK

13.2.1 Analysis on Secondary Endpoints

For the change from baseline in total dietary protein intake, the observed values, change and percent change from baseline at each visit will be summarized by treatment group. Similar summary will also be provided for protein intake from medical food and from intact food

separately. The values collected on the three-day diet diary will be averaged for each parameter at each visit before being summarized.

In Part 2, change and percent change in dietary protein intake from average of Week 69 and Week 73 visits will also be summarized for participants transitioning from control (diet-only) treatment arm to pegvaliase. For participants continuing pegvaliase in Part 2, change and percent change in dietary protein intake from Part 1 baseline will be summarized for data collected in Part 2.

For plasma sample of trough PK, table summary and subject listing of observed value will be provided at each visit. The PK parameters derived from the intensive PK samples will also be summarized. Details of PK analysis of pegvaliase concentration data will be provided in a separate Clinical Pharmacology analysis plan.

13.3 Exploratory Endpoints

The exploratory endpoints are as follows:

- The ADHD Rating Scale IV inattention subscale (ADHD RS-IV) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study
- The Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2) rated by the parent/guardian and performed at Baseline (Day 1, pre-dose) and every 12 weeks throughout the study
- Change from baseline (or first administration) in Phenylketonuria Symptom Severity and Impacts Scale (PKU-SSIS) Version 2.0 scores
- Change from baseline (or first administration) in global severity items (Patient Global Impression of Severity [PGIS], Caregiver Global Impression of Severity [CaGIS], and Clinician Global Impression of Severity [CGIS])
- Change in global change items (Patient Global Impression of Change [PGIC] and Caregiver Global Impression of Change [CaGIC])
- Time to participants' optimal maintenance dose
- Number of patients who require the highest (60mg/day) maintenance dose
- Percentage of patients in the blood Phe ranges of less than 600 µmol/L and less than 360 µmol/L, respectively

13.3.1 Exploratory Clinical Outcomes Assessment Measures

13.3.1.1 The ADHD Rating Scale IV (ADHD RS-IV) Inattention Subscale

The ADHD RS-IV instrument is completed independently by the parent/guardian. The same parent/guardian must complete the scale at each scheduled time point. The instrument consists of 2 nine-item scales that evaluate symptom severity for inattention and hyperactivity/impulsivity, respectively, during the previous month. Each scale is scored separately. Each item is rated on a 4-point severity scale (0, none; 1, mild; 2, moderate; 3, severe); scores of 2 or 3 on an individual item indicate a symptom.

ADHD RS Inattention (IA) subscale is the sum of the 9 odd-numbered items.

ADHD RS Hyperactivity-Impulsivity (HI) subscale is the sum of the 9 even-numbered items

ADHD RS total score will be the sum of ADHD RS IA and ADHD RS HI

13.3.1.2 Behavior Rating Inventory of Executive Function, Second Edition (BRIEF2)

The BRIEF2 is a 63-item measure in 8 non-overlapping clinical scales and 2 validity scales. It is designed to assess executive function skills in children 5 to 18 years of age (Gioia 2002). The same parent/guardian must complete the BRIEF2 at each scheduled time point. These scales form 2 indexes: (1) Behavior Regulation (3 scales) and (2) Metacognition (5 scales), as well as a Global Executive Composite score which takes into account all of the clinical scales and represents the adolescent's overall executive function.

Behavior Regulation Index (BRI), Emotion Regulation Index (ERI), Cognitive Regulation Index (CRI) and Global Executive Composite (GEC) will be summarized, and their definition are as follows:

BRI = Inhibit + Self-Monitor

ERI = Shift + Emotional Control

CRI = Initiate + Working Memory + Plan/Organize + Task-Monitor + Organization of Materials

GEC = BRI + ERI + CRI

13.3.1.3 PKU Symptom Severity and Impacts Scale (PKU-SSIS) v2.0 – Adolescent Version

The PKU Symptom Severity and Impact Scale (PKU-SSIS) was designed to evaluate neuropsychological symptoms and impacts in early-treated patients with PKU. The PKU-SSIS is comprised of 22 items that constitute 3 symptoms domains: 1) Emotional, Mood and

Psychological; 2) (Neuro)cognitive, Executive, and Intellectual Function; 3) Physical Health. It also has 4 impact domains: 1) Social Relations; 2) Level of Independence; 3) General Wellbeing; and 4) Self-care (Quinn 2022). The instrument total score is compiled by summing the domain scores and converting to a percentage score (ie, Total score = (summed score/88)*100). Participants will complete the PKU-SSIS as indicated in the SoA.

13.3.1.4 Global Anchor Measures (Caregiver Global Impression of Severity [CaGIS], Caregiver Global Impression of Change [CaGIC], Patient Global Impression of Severity [PGIS], Patient Global Impression of Change [PGIC], and Clinician Global Impression of Severity [CGIS]

Global impression measures for severity and change (or global anchors) are standard items that have been widely used across a broad range of disease areas and are typically included in clinical trials as anchor measures to help establish meaningful change in other COAs.

Global impression of severity anchor measures (for Caregivers and Patients) rate the overall disease severity using a 5-point Likert scale from "None/Not at all impacted" to "Very severe/Very severe impact." Global impression of change anchor measures (for Caregivers, Participants, and Clinicians) assess if there has been an improvement or change in clinical status using a 5-point Likert scale from "Much better" to "Much worse.".

13.3.1.5 Analysis of Exploratory Clinical Outcomes Assessment Endpoints

Descriptive summary will be provided for all exploratory endpoints by treatment group. ADHD RS-IV, BRIEF2, PKU-SSIS, and global anchor measures will be summarized at respective scheduled visits. Scale/domain scores and total scores for ADHD RS-IV, BRIEF2, and PKU-SSIS will be reported by timepoint. Change from baseline for ADHD RS-IV scores BRIEF2 scores and PKU-SSIS scores (by scale/domain and total scores for all three measures) will be summarized at each visit. Change from baseline for ADHD RS-IV scores will also be summarized for subjects with ADHD RS-IV IA and HI subscale score ≥ 9 at baseline. In addition, ANCOVA models will be performed, which will include baseline measurements (when available) as covariates, randomization strata (baseline Phe ≤ 1000 µmol/L or > 1000 µmol/L) and treatment group as factors. The least square (LS) mean and 95% confidence interval will be calculated for the treatment difference between pegvaliase and diet-only arms following 72 weeks on study. In Part 2, change scores from Week 73 visit will also be summarized for participants transitioning from control to pegvaliase.

13.3.2 Other Exploratory Analyses

13.3.2.1 Sustained Blood Phe Reductions

Sustained blood Phe reductions will be evaluated by sustained Phe response (SPR) with a longitudinal model, where a nonparametric smoother is used to capture the expected value of blood Phe at time *t*, $E(Phe_t)$, given adjacent or local blood Phe measures, and a 95% confidence band for the smoother estimate of $E(Phe_t)$ will be calculated. The confidence band represents the range of plausible expected blood Phe values for any arbitrary point in time (interpolating over the time series), given locally retrospective and prospective information.

SPR is considered to have been achieved when the upper bound of the confidence band was below each of blood Phe concentration thresholds (120, 360, and 600 µmol/L).

Time spent in duration of SPR ≤ 120, 360, and 600 µmol/L and time to the first SPR ≤ 120, 360, and 600 µmol/L will be summarized by treatment arm.

13.3.2.2 Blood Phe within Target Range

Duration and percentage of time with blood Phe ≤ 120, 360, and 600 µmol/L during Part 1 will be calculated for each subject as follows:

1) For blood Phe measurements collected after week 1 and up to week 73, identify the study visits with blood Phe in the target range (≤ 120, 360 or 600 µmol/L)
2) For each visit identified in step 1), calculate duration of segment (in weeks) as study week of the current visit – study week of the previous visit
3) Calculate the total **duration of blood Phe in target range** as sum of duration of segments derived in step 2)
4) **Percentage of time in target range** is defined as total duration of blood Phe in target range / total follow-up time in Part 1

Descriptive summary will be provided for duration and percentage of time with blood Phe in target range by treatment arm

AUC and average blood Phe in target range (≤ 120, 360 or 600 µmol/L) during Part 1 will be calculated for each subject as follows:

5) For each visit identified in step 1), calculate the area of the segment as duration of the segment (derived in step 2) × the associated blood Phe value
6) **AUC in target range** is defined as sum of the area of segments derived in step 5)

7) **Average blood Phe in target range** is defined as AUC in target range derived in step 6 / total duration of blood Phe in target range derived in step 3).

Descriptive summary will be provided for AUC and average blood Phe in target range ≤ 120, 360, and 600 µmol/L by treatment arm.

13.4 Examination of Efficacy by Subgroups

The following subgroup analysis will be performed on the primary endpoint:

- Baseline phe: ≤ 1000 µmol/L or > 1000 µmol/L
- Sex
- Baseline Age (12 to < 16, 16 to < 18)
- Country

14 SAFETY EVALUATIONS

Safety will be assessed by examining the incidence, exposure adjusted event rate, severity grade, and relationship to study drug of all treatment-emergent adverse events (TEAEs) reported during the study period. In addition, clinical laboratory results, vital signs, pregnancy test, physical exam and ECG values will be assessed. In general, two sets of safety analyses will be performed: Part 1 period and combined Part 1 and 2 periods. AE data will be summarized by the actual treatment received at the time of AE onset (pegvaliase or diet-only).

14.1 Adverse Events

AEs will be coded in accordance with the MedDRA (version 24.1). Only treatment-emergent adverse events (TEAEs) occurring and reported during the study period will be included in adverse event summaries. A TEAE is defined as any AE that newly appeared or worsened in severity after first dose of pegvaliase (pegvaliase arm) or on or after study day 1 (diet-only arm) until 30 days after last dose of the study.

In Part 2 participants transitioning from control to pegvaliase, a TEAE occurred during pegvaliase treatment period is defined as any AE that appeared or worsened in severity after first dose of pegvaliase until 30 days after last dose.

14.1.1 All Adverse Events

The incidence and frequency of all treatment-emergent AEs will be summarized by system organ class (SOC), preferred term (PT), relationship to study drug and National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) severity grades.

For AEs that occurred more than once during the study, the maximum severity will be used to summarize the AEs by severity.

If the onset date or end date of an adverse event is partial, the same imputation rules described in Section 2.6 will be applied.

The following summary will be performed for treatment-emergent AEs:

- All AEs by SOC and PT
- All AEs by SOC, PT and severity
- AEs assessed by investigators as related to study drug by SOC and PT
- AEs assessed by investigators as related to study drug by SOC, PT and severity
- Serious AEs (SAEs) by SOC and PT
- All AEs by PT in descending frequency order
- All AEs by SOC and PT with exposure adjusted event rate
- AEs leading to withdrawal from study by SOC and PT
- AEs leading to study drug discontinuation by SOC and PT

In addition, summary tables and listings will be provided for AEs of special interest:

- Acute systemic hypersensitivity reaction (refer to **Appendix 1**)
- Anaphylaxis per FDA criteria (refer to **Appendix 1**)
- Anaphylaxis per NIAID/FAAN criteria
- Angioedema per FDA criteria
- Angioedema per sponsor criteria
- Serum sickness-like reaction
- Hypersensitivity reaction (EU)
- Hypersensitivity reaction (US)
- Injection site reaction
- Injection site skin reaction ≥ 14 days duration
- Generalized skin reaction ≥ 14 days duration
- Severe injection site reaction defined as an injection site reaction that is considered CTCAE Grade ≥ 3

- Hypophenylalaninemia events (defined as blood Phe < 30 µmol/L on 2 consecutive measurements)
- Arthralgia that is either persistent (AE duration ≥ 6 months), severe (CTCAE Grade ≥ 3), or that requires treatment with a steroid (excluding topical)
- Arthralgia (sponsor-defined)
- Neuropsychiatric disorder
- Hypersensitivity AEs
- Arthralgia /Arthritis
- Lymphadenopathy ≥ 14 Days Duration
- Persistent Urticaria ≥ 14 Days Duration
- Infection and Infestations ≥ 30 Days Duration
- Ischemic Heart Disease
- Proteinuria/Albuminuria
- Hematuria
- Blood Creatinine Increased
- Renal Impairment
- Increased liver function test or Events Involving Hepatic Impairment
- Anemia
- Thrombocytopenia
- Hemolysis
- Myalgia
- Serositis
- Potential central nervous system vascular complications

14.2 Clinical Laboratory Tests

Descriptive statistics will be presented for clinical laboratory tests at each scheduled visit and for change in laboratory values from baseline. The proportion of participants who experienced at least one laboratory test result outside the normal ranges will be presented for each measurement as appropriate. Shift table from baseline to most extreme post-baseline value based on normal range and based on CTCAE grading (where available) will also be generated for each parameter as appropriate. A subject listing of laboratory test results for

hematology, chemistry, urinalysis, and other laboratory tests will be provided separately. Values with CTCAE Grade ≥ 3 will be flagged in the listings.

A listing of laboratory test results for participants with alanine transaminase (ALT) or alanine aminotransferase (AST) ≥ 3x ULN and total bilirubin ≥ 2x ULN will also be provided.

14.3 Vital Signs

Vital signs will include systolic blood pressure (SBP) and diastolic blood pressure (DBP) measured in millimeters of mercury (mm Hg), heart rate in beats per minute, respiration rate in breaths per minute, and temperature in degrees Celsius (°C).

Summary statistics including n, mean, SD, median, minimum, and maximum will be provided for these parameters at each visit. A subject listing will also be provided.

14.4 12-lead Electrocardiogram

The frequency of abnormalities and clinically significant abnormalities in 12-lead electrocardiogram will be summarized at each visit. A subject listing of ECG findings will be provided.

14.5 Physical Examination

Physical examinations will include assessments of general appearance; head, eyes, ears, nose, and throat; the cardiovascular, dermatologic, lymphatic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Other body systems may be examined if needed. A subject listing for clinically significant findings at each visit will be provided.

14.6 Pregnancy Test

A subject listing for pregnancy urine sample test at each visit will be provided.

15 IMMUNOGENICITY TESTS

Immunogenicity will be performed using validated immunogenicity assays. Immunogenicity assays include total anti-pegvaliase antibodies (TAb), anti-PAL IgG, anti-PAL IgM, anti-PEG IgM, anti-PEG IgG, and neutralizing antibodies (NAb). In the event of a Hypersensitivity Reaction visit (HRV), anti-pegvaliase IgE will be assessed.

For immunogenicity analysis, incidence tables and titer summary statistics including mean, median, standard deviation, and minimum/maximum titer values at each study visit will be provided for each antibody analyte.

Anti-drug antibody impact on safety will be evaluated. Plots and tables will be generated to explore the potential impact of anti-drug antibodies on Hypersensitivity Reaction (US and EU) frequency and severity. A listing will be provided for each subject who has Hypersensitivity Reaction Visit.

Anti-drug antibody impact on efficacy will also be evaluated. Plots and tables may be generated to explore the potential impact of anti-drug antibodies on blood Phe levels.

16 APPENDICES

Appendix 1: Search Strategies for Adverse Event of Special Interest, and Sponsor Defined AEs of Clinical Significance

MedDRA version: most current version at program termination

1. Anaphylaxis per NIAID/FAAN criteria

Anaphylaxis per NIAID/FAAN is defined using the NIAID/FAAN clinical diagnostic criteria through review on the potential anaphylaxis per NIAID/FAAN cases, which are identified using Broad algorithmic anaphylactic reaction SMQ with the below algorithm:

Algorithm = A or (B and C) or (D and (B or C))

Name of Search	Category	Preferred Term
Broad Algorithmic Anaphylactic Reaction SMQ	A	Anaphylactic reaction
	A	Anaphylactic shock
	A	Anaphylactic transfusion reaction
	A	Anaphylactoid reaction
	A	Anaphylactoid shock
	A	Circulatory collapse
	A	Dialysis membrane reaction
	A	Kounis syndrome
	A	Procedural shock
	A	Shock
	A	Shock symptom
	A	Type I hypersensitivity
	B	Acute respiratory failure
	B	Asthma
	B	Bronchial oedema
	B	Bronchospasm
	B	Cardio-respiratory distress
	B	Chest discomfort
	B	Choking
	B	Choking sensation
	B	Circumoral oedema
	B	Cough
	B	Cough variant asthma

Name of Search	Category	Preferred Term
	B	Cyanosis
	B	Dyspnoea
	B	Hyperventilation
	B	Irregular breathing
	B	Laryngeal dyspnoea
	B	Laryngeal oedema
	B	Laryngospasm
	B	Laryngotracheal oedema
	B	Mouth swelling
	B	Nasal obstruction
	B	Oedema mouth
	B	Oropharyngeal oedema
	B	Oropharyngeal spasm
	B	Oropharyngeal swelling
	B	Pharyngeal oedema
	B	Pharyngeal swelling
	B	Respiratory arrest
	B	Respiratory distress
	B	Respiratory failure
	B	Reversible airways obstruction
	B	Sensation of foreign body
	B	Sneezing
	B	Stridor
	B	Swollen tongue
	B	Tachypnoea
	B	Throat tightness
	B	Tongue oedema
	B	Tracheal obstruction
	B	Tracheal oedema
	B	Upper airway obstruction
	B	Vaccine associated enhanced respiratory disease
	B	Wheezing
	C	Allergic oedema
	C	Angioedema
	C	Circumoral swelling
	C	Erythema

Name of Search	Category	Preferred Term
	C	Eye oedema
	C	Eye pruritus
	C	Eye swelling
	C	Eyelid oedema
	C	Face oedema
	C	Flushing
	C	Injection site urticaria
	C	Lip oedema
	C	Lip swelling
	C	Nodular rash
	C	Ocular hyperaemia
	C	Oedema
	C	Oedema blister
	C	Periorbital oedema
	C	Periorbital swelling
	C	Pruritus
	C	Pruritus allergic
	C	Rash
	C	Rash erythematous
	C	Rash pruritic
	C	Skin swelling
	C	Swelling
	C	Swelling face
	C	Swelling of eyelid
	C	Urticaria
	C	Urticaria papular
	D	Blood pressure decreased
	D	Blood pressure diastolic decreased
	D	Blood pressure systolic decreased
	D	Cardiac arrest
	D	Cardio-respiratory arrest
	D	Cardiovascular insufficiency
	D	Diastolic hypotension
	D	Hypotension
	D	Hypotensive crisis
	D	Post procedural hypotension

2. Anaphylaxis per FDA criteria

Anaphylaxis events defined according to the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) methodology are referred to as "anaphylaxis per FDA criteria". The FDA's assessment methodology includes individual case safety reports with any of the following characteristics:

- Clinical manifestations consistent with National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) Criterion #1 (skin involvement + respiratory or cardiovascular compromise); or
- Verbatim reports of anaphylaxis/anaphylactic reaction (or synonyms), even if NIAID/FAAN criteria were not met; or
- Cases involving subject injection of epinephrine alone, even if subject did not experience signs/symptoms necessary to meet NIAID/FAAN criteria.

3. Acute systemic hypersensitivity reaction

An acute systemic hypersensitivity reaction (referred to as anaphylaxis in the protocol) is defined as an episode meeting National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network (NIAID/FAAN) criteria according to an internal BioMarin assessment performed by the Internal Data Safety Review Board. The acute systemic hypersensitivity reaction events will also be presented if they meet Brown's severe criteria as determined by the Internal Safety Review Board.

4. Angioedema per FDA criteria

Angioedema as an AE reported as any PT in the MedDRA Angioedema Higher Level Term excluding idiopathic angioedema, Gleich's syndrome, and hereditary angioedema (see PT term list below:

Angioedema
Circumoral oedema
Eyelid oedema
Face oedema
Intestinal angioedema
Laryngeal oedema
Laryngotracheal oedema
Lip oedema
Lip swelling
Mouth swelling

Oculorespiratory syndrome
Oedema mouth
Oropharyngeal swelling
Periorbital oedema
Pharyngeal oedema
Swelling face
Swollen tongue
Tongue oedema

and also excluding any AE which is part of an Anaphylaxis per FDA criteria episode.

5. Angioedema per sponsor criteria

A 2-step process to identify angioedema per sponsor criteria, the following assessment was performed on the entire AE dataset:

- Step 1: all reported ADRs suggestive of angioedema using the Angioedema MedDRA HLT search, which includes the following 21 PTs: angioedema, circumoral oedema, eyelid oedema, face oedema, Gleich's syndrome, hereditary angioedema, idiopathic angioedema, intestinal angioedema, laryngeal oedema, laryngotracheal oedema, lip oedema, lip swelling, mouth swelling, oculorespiratory syndrome, oedema mouth, oropharyngeal swelling, periorbital oedema, pharyngeal oedema, swelling face, swollen tongue, and tongue oedema.
- Step 2: BioMarin then clinically assessed the retrieved data identified from Step 1 to assign concurrent events into episodes and to exclude:
    - False positive ADR reports that have clear confounders which impact assessment, e.g., onset of event(s) $\geq$ 30 days after last dose or events/episodes with a very clear alternative etiology, such as throat swelling secondary to intubation). Cases were not excluded where the events have a long duration which may be atypical for angioedema, or where events occurred concurrent with other conditions which may have contributed to their development (e.g., eye swelling reported during episode(s) of seasonal allergy).
- Episodes which occurred as part of an acute systemic hypersensitivity reaction and, therefore, will already be included in the incidence for acute systemic hypersensitivity reactions (to avoid duplication).

In the sponsor's opinion, the methodology described above is more likely to be reflective of the true incidence of angioedema manifestations.

6. Serum Sickness-like reaction

Serum sickness-like reaction will be defined by including all preferred term of serum sickness, serum sickness-like reactions and Type III immune complex mediated reactions.

7. Hypersensitivity Reaction (EU)

Hypersensitivity reactions are defined as the modified hypersensitivity SMQ (narrow SMQ) or an anaphylaxis event as described in Appendix 1 - Listing 1 and adjudicated as per the Acute Systemic Hypersensitivity Reaction in section 10.5.2 in Protocol

The following terms should be excluded from the Modified Hypersensitivity (Narrow SMQ) search criteria:

injection site rash

injection site urticaria

8. Hypersensitivity Reaction (US)

Hypersensitivity reaction is defined as the modified hypersensitivity SMQ (narrow SMQ), plus the anaphylaxis adjudicated by FDA criteria.

The following terms should be excluded from the Modified Hypersensitivity (Narrow SMQ) search criteria:

injection site rash

injection site urticaria

anaphylactic reaction

anaphylactoid reaction

9. Injection site reaction

Injection-site reaction will be identified using injection site reaction Higher Level MedDRA term.

10. Severe Injection-site reaction

Severe injection site reaction will be identified as any injection site reaction report that is reported as Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or above in severity and meeting MedDRA high level term (HLT) for Injection site reaction.

11. Injection site skin reaction ≥ 14 days duration

Injection site skin reaction with ≥ 14 days duration will be identified by preferred term search using the list below. To be included as injection site skin reaction, the duration of the event needs to be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Injection site skin reaction	Injection site anaesthesia
	Injection site extravasation
	Injection site phlebitis
	Injection site movement impairment
	Injection site lymphadenopathy
	Injection site atrophy
	Injection site cyst
	Injection site erosion
	Injection site fibrosis
	Injection site granuloma
	Injection site induration
	Injection site mass
	Injection site necrosis
	Injection site oedema
	Injection site ulcer
	Injection site hypersensitivity
	Injection site irritation
	Injection site discomfort
	Injection site dysaesthesia
	Injection site hyperaesthesia
	Injection site hypoaesthesia
	Injection site swelling
	Injection site calcification
	Injection site pustule
	Injection site nodule

Name of Search	Preferred Term
	Injection site scar
	Injection site bruising
	Injection site haematoma
	Injection site haemorrhage
	Injection site hypertrophy
	Injection site pain
	Injection site paraesthesia
	Injection site pruritus
	Injection site urticaria
	Injection site papule
	Injection site macule
	Injection site vasculitis
	Injection site plaque
	Injection site discolouration
	Injection site injury
	Injection site abscess
	Injection site abscess sterile
	Injection site dermatitis
	Injection site erythema
	Injection site infection
	Injection site inflammation
	Injection site rash
	Injection site reaction
	Injection site thrombosis
	Injection site vesicles
	Injection site ischaemia
	Injection site cellulitis
	Injection site discharge
	Injection site scab
	Injection site eczema
	Injection site recall reaction
	Injection site exfoliation

12. Generalized skin reaction ≥ 14 days duration

Generalized skin reaction ≥ 14 days duration will be identified in following ways:

- Preferred term search using below listing, or

- Broad vasculitis SMQ

To be included as generalized skin reaction, the duration of the event needs to be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Generalized Skin Reaction	Acute cutaneous lupus erythematosus
	Acute febrile neutrophilic dermatosis
	Acute generalised exanthematous pustulosis
	Angiodermatitis
	Angioedema
	Angular cheilitis
	Atypical fibroxanthoma
	Atypical lymphocytic lobular panniculitis
	Autoimmune dermatitis
	Benign neoplasm of skin
	Blister
	Blister infected
	Blister rupture
	Blood blister
	Capillaritis
	Carbuncle
	Cellulitis
	Cellulitis enterococcal
	Cellulitis gangrenous
	Cellulitis staphylococcal
	Cellulitis streptococcal
	Cheilitis
	Cheilitis granulomatosa
	Cheilosis
	Chronic cutaneous lupus erythematosus
	Chronic papillomatous dermatitis
	Chronic pigmented purpura
	Circumoral oedema
	Coma blister
	CREST syndrome
	Cutaneous calcification

	Cutaneous lupus erythematosus
	Cutaneous vasculitis
	Cutaneovisceral angiomatosis with thrombocytopenia
	Dermatitis
	Dermatitis allergic
	Dermatitis atopic
	Dermatitis bullous
	Dermatitis exfoliative
	Dermatitis exfoliative generalised
	Dermatitis infected
	Dermatitis psoriasiform
	Drug eruption
	Drug reaction with eosinophilia and systemic symptoms
	Dry gangrene
	Dry skin
	Ecchymosis
	Eczema
	Eczema nummular
	Eczema vesicular
	Eosinophilic panniculitis
	Epidermal necrosis
	Epidermolysis
	Epidermolysis bullosa
	Eruptive pseudoangiomatosis
	Erythema
	Erythema elevatum diutinum
	Erythema induratum
	Erythema infectiosum
	Erythema migrans
	Erythema multiforme
	Erythema nodosum
	Excessive granulation tissue
	Excoriation
	Exfoliative rash
	Eyelid oedema
	Face oedema
	Fixed eruption

	Furuncle
	Gangrene
	Granuloma
	Granuloma annulare
	Granuloma skin
	Granulomatous dermatitis
	Haemorrhage subcutaneous
	Haemorrhage subepidermal
	Haemorrhagic urticaria
	Haemorrhagic vasculitis
	Hand dermatitis
	Henoch-Schonlein purpura
	Hypersensitivity vasculitis
	Hypertrophic scar
	Idiopathic angioedema
	Idiopathic urticaria
	Infected dermal cyst
	Infected skin ulcer
	Interstitial granulolmatous dermatitis
	Itching scar
	Jaundice
	Keratolysis exfoliativa acquired
	Laryngeal oedema
	Laryngotracheal oedema
	Lichenification
	Lip blister
	Lip oedema
	Lip swelling
	Lipoatrophy
	Lipohypertrophy
	Livedo reticularis
	Lupus vulgaris
	Lupus-like syndrome
	Macule
	Medical device site laceration
	Morphoea
	Mouth swelling

	Mucocutaneous rash
	Mucocutaneous ulceration
	Necrobiosis lipoidica diabeticorum
	Necrotising panniculitis
	Neoplasm skin
	Neurodermatitis
	Nodular vasculitis
	Oedema mouth
	Oropharyngeal swelling
	Osler's nodes
	Overlap syndrome
	Palisaded neutrophilic granulomatous dermatitis
	Palmar erythema
	Palpable purpura
	Panniculitis
	Panniculitis lobular
	Papule
	Pemphigoid
	Pemphigus
	Periorbital oedema
	Perivascular dermatitis
	Petechiae
	Pharyngeal oedema
	Pharyngeal swelling
	Pigmentation disorder
	Pruritus
	Pruritus allergic
	Pruritus generalised
	Psoriasis
	Psoriatic arthropathy
	Purpura
	Purpura fulminans
	Pustular psoriasis
	Pyoderma
	Rash
	Rash erythematous
	Rash follicular

	Rash generalised
	Rash macular
	Rash maculo-papular
	Rash maculovesicular
	Rash morbilliform
	Rash papular
	Rash papulosquamous
	Rash pruritic
	Rash pustular
	Rash rubelliform
	Rash vesicular
	Recall phenomenon
	Red man syndrome
	Rheumatoid nodule
	Scar
	Sclerodactylia
	Scleroderma
	Scleroedema
	Seborrhoea
	Seborrhoeic dermatitis
	Septal panniculitis
	Skin atrophy
	Skin disorder
	Skin dystrophy
	Skin erosion
	Skin exfoliation
	Skin fibrosis
	Skin haemorrhage
	Skin hyperpigmentation
	Skin hyperplasia
	Skin hypertrophy
	Skin hypopigmentation
	Skin hypoplasia
	Skin induration
	Skin infection
	Skin irritation
	Skin lesion

	Skin maceration
	Skin mass
	Skin necrosis
	Skin oedema
	Skin plaque
	Skin reaction
	Skin swelling
	Skin tightness
	Skin toxicity
	Skin ulcer
	Skin ulcer haemorrhage
	Skin wound
	Stevens-Johnson syndrome
	Subacute cutaneous lupus erythematosus
	Subcutaneous abscess
	Subcutaneous haematoma
	Swelling face
	Swelling of eyelid
	Swollen tongue
	Systemic lupus erythematosus
	Systemic lupus erythematosus rash
	Systemic sclerosis
	Thrombocytopenic purpura
	Thrombotic thrombocytopenic purpura
	Tongue oedema
	Toxic epidermal necrolysis
	Toxic skin eruption
	Urticaria
	Urticaria chronic
	Urticaria papular
	Vascular purpura
	Vascular skin disorder
	Vasculitic rash
	Vasculitic ulcer
	Weber-Christian disease
	Xanthogranuloma
	Xanthoma

13. Arthralgia (sponsor defined)

Arthralgia combines preferred term of arthralgia, pain in extremity, back pain, musculoskeletal pain, neck pain.

14. Arthralgia (steroid treated)

To identify the events of arthralgia that were treated with steroids (excluding topicals), the following steps are required.

- Identify the events of arthralgia (Arthralgia combines the preferred terms: of arthralgia, pain in extremity, back pain, musculoskeletal pain, neck pain), and Identify those arthralgia AEs that were treated with a concomitant medication after the onset date of the start of the arthralgia AE and before the resolution date of the arthralgia AE; and
- The resultant file will be clinically reviewed by BioMarin to identify the arthralgia events that were treated with a concomitant steroid (excluding those events that were administered topically, or where the steroid medication was used during an arthralgia episode but for an indication other than arthralgia).

15. Severe or Persistent (≥ 6 months) arthralgia

Arthralgia will be identified as the preferred terms of arthralgia, pain in extremity, back pain, musculoskeletal pain, and neck pain. Persistent arthralgia is defined as any arthralgia event that has a duration of the event ≥ 6 months (Multiple AE records with same reported term and ≤ 1 day difference between the start date and end date are considered as same event). Severe arthralgia is defined as any arthralgia event that is reported as CTCAE Grade 3 or above in severity.

16. Hypophenylalaninemia

Hypophenylalaninemia is defined as a blood phenylalanine (Phe) level ≤ 30 µmol/L for a minimum of 2 consecutive values, with the start of the event defined as the first blood Phe assessment day with < 30 µmol/L and the end of the event defined as the day prior to the first blood Phe assessment ≥ 30 µmol/L.

17: Neuropsychiatric disorder

A neuropsychiatric disorder is defined as Psychiatric disorder SOC + modified Nervous system SOC.

Name of Search	Preferred Term
Modified nervous SOC	Acalculia
	Agitation postoperative
	Agnosia
	Agraphia
	AIDS dementia complex
	Akathisia
	Altered state of consciousness
	Amnesia
	Amnestic disorder
	Anosognosia
	Anterograde amnesia
	Aphasia
	Apraxia
	Aura
	Autism
	Borderline mental impairment
	Circadian rhythm sleep disorder
	Clumsiness
	Cognitive disorder
	Coprolalia
	Crying
	Decreased eye contact
	Delayed sleep phase
	Dementia
	Depressed level of consciousness
	Disorganised speech
	Disorientation
	Disturbance in attention
	Dreamy state
	Dysphemia
	Dysphonia psychogenic
	Dyssomnia
	Echolalia
	Fine motor skill dysfunction
	Formication

	Fumbling
	Hyperkinesia
	Hypersomnia
	Hypokinesia
	Hyporesponsive to stimuli
	Hyposomnia
	Incoherent
	Initial insomnia
	Insomnia
	Intelligence increased
	Irregular sleep phase
	Judgement impaired
	Lethargy
	Memory impairment
	Mental impairment
	Mental retardation
	Mild mental retardation
	Moderate mental retardation
	Mutism
	Neurological decompensation
	Neurological symptom
	Parosmia
	Phantom pain
	Poor quality sleep
	Posturing
	Presenile dementia
	Profound mental retardation
	Psychomotor hyperactivity
	Psychomotor skills impaired
	Repetitive speech
	Restlessness
	Sedation
	Senile dementia
	Sleep phase rhythm disturbance
	Slow response to stimuli
	Slow speech

	Somnolence
	Speech disorder
	Stereotypy
	Stupor
	Sudden onset of sleep
	Symbolic dysfunction
	Unresponsive to stimuli
	Verbigeration

18: Hypersensitivity AEs

Hypersensitivity AEs will be identified in two ways:

- Broad Algorithmic anaphylactic reaction Standardized MedDRA Queries (SMQ) with the below algorithm:

  Algorithm = A or (B and C) or (D and (B or C))

- Modified Hypersensitivity SMQ to include below additional preferred terms

Name of Search	Preferred Term
Additional preferred terms for Modified Hypersensitivity SMQ	Arthralgia
	Arthritis
	Eye inflammation
	Eye irritation
	Eye pain
	Joint stiffness
	Joint swelling
	Pyrexia
	Vision blurred
	Polyarthritis

19: Arthralgia /Arthritis:

Arthralgia combines preferred term of arthralgia, Joint crepitation, Joint effusion, Joint destruction, Joint noise, Joint swelling, Joint stiffness.

Arthritis was identified using following preferred terms:

Name of Search	Preferred Term
Arthritis	Amyloid arthropathy
	Antithyroid arthritis syndrome
	Arthritis
	Arthritis allergic
	Arthritis climacteric
	Arthritis enteropathic
	Arthritis reactive
	Arthropathy
	Arthrotoxicity
	Autoimmune arthritis
	Behcet's syndrome
	Blau syndrome
	Carcinomatous polyarthritis
	Diabetic arthropathy
	Haemarthrosis
	Haemophilic arthropathy
	Hyper IgD syndrome
	Injection site joint inflammation
	Monarthritis
	Multicentric reticulohistiocytosis
	Neuropathic arthropathy
	Palindromic rheumatism
	Polyarthritis
	Poncet's disease
	Pyogenic sterile arthritis pyoderma gangrenosum and acne syndrome
	Reiter's syndrome
	Rheumatic fever
	Sacroiliitis
	Seronegative arthritis
	SLE arthritis
	Sympathetic posterior cervical syndrome
	Traumatic arthritis
	Traumatic arthropathy

20: Lymphadenopathy ≥ 14 Days Duration

Lymphadenopathy will be identified from preferred terms outlined below. To be included in the results, the duration of the event should be ≥ 14 days (Multiple AE records with same preferred term and ≤ 1 day difference between the start date and end date and with severity worsens are considered as same event).

Name of Search	Preferred Term
Lymphadenopathy	Abdominal lymphadenopathy
	Hilar lymphadenopathy
	Injection site lymphadenopathy
	Lymph node calcification
	Lymph node fibrosis
	Lymph node pain
	Lymphadenitis
	Lymphadenocyst
	Lymphadenopathy
	Lymphadenopathy mediastinal
	Lymphatic disorder
	Lymphoid tissue hyperplasia
	Paratracheal lymphadenopathy
	Persistent generalised lymphadenopathy
	Retroperitoneal lymphadenopathy
	Spleen disorder
	Splenomegaly
	lymph node palpable

21: Persistent Urticaria ≥ 14 Days Duration

Persistent urticaria will be identified using preferred term: urticaria, with event duration ≥ 14 days (Multiple AE records with same reported term and ≤ 1day difference between the start date and end date and with severity worsens were considered as same event).

22: Infection and Infestations ≥ 30 Days Duration

Infection and Infestations will be identified using Infection and Infestations SOC. To be included in the result, the duration of the event should be ≥ 30 days (Multiple AE records with same reported term and ≤ 1day difference between the start date and end date and with severity worsens were considered as same event).

23: Ischemic Heart Disease

Ischemic heart disease will be identified using myocardial infarction narrow SMQ + Other Ischemic heart disease SMQ

24: Proteinuria/Albuminuria

Proteinuria/Albuminuria will be identified using the modified Proteinuria SMQ (narrowed SMQ adding a couple of the terms from the broad SMQ).

Name of Search	Preferred Term
Proteinuria/Albuminuria	Albumin globulin ratio increased
	Albumin urine present
	Albuminuria
	Bence Jones protein urine present
	Bence Jones proteinuria
	Beta 2 microglobulin urine increased
	Globulinuria
	Microalbuminuria
	Myoglobinuria
	Orthostatic proteinuria
	Protein urine
	Protein urine present
	Proteinuria
	Urine albumin/creatinine ratio abnormal
	Urine albumin/creatinine ratio increased
	Urine protein/creatinine ratio abnormal
	Urine protein/creatinine ratio increased
	Aminoaciduria
	Haemoglobinuria

25: Hematuria

Hematuria was identified using following preferred terms:

Name of Search	Preferred Term
Hematuria	Haematuria
	Haemoglobin urine present
	Haemoglobin urine
	Haemoglobinuria
	Red blood cells urine positive
	Red blood cells urine
	Blood urine present
	Blood urine

26: Blood Creatinine Increased

Blood creatinine increased will be identified using the following preferred terms: Blood creatinine abnormal, blood creatinine increased.

27: Renal Impairment

Renal impairment will be identified using a combination of modified narrow tubulointerstitial diseases SMQ, modified narrow acute renal failure SMQ, and modified narrow chronic kidney disease SMQ.

Name of Search	Preferred Term
Modified narrow tubulointerstitial diseases (SMQ)	Acidosis hyperchloraemic
	Acquired aminoaciduria
	Acute phosphate nephropathy
	Aminoaciduria
	Beta-N-acetyl-D-glucosaminidase increased
	Crystal nephropathy
	Eosinophils urine present
	Fanconi syndrome acquired
	Hyperphosphaturia
	Isosthenuria
	Nephritis allergic
	Nephrogenic diabetes insipidus
	Nephropathy toxic
	Renal glycosuria
	Renal papillary necrosis
	Renal tubular acidosis

	Renal tubular atrophy
	Renal tubular disorder
	Tubulointerstitial nephritis
	Tubulointerstitial nephritis and uveitis syndrome
	Urine phosphorus increased
	Urine retinol binding protein increased
	Albumin urine present
	Albuminuria
	Cylindruria
	Haematuria
	Haemoglobin urine present
	Haemoglobinuria
	Microalbuminuria
	Protein urine present
	Proteinuria
	Red blood cells urine positive
	Renal tubular necrosis
	Sterile pyuria

Name of Search	Preferred Term
Modified narrow acute renal failure (SMQ)	Acute kidney injury
	Acute phosphate nephropathy
	Acute prerenal failure
	Anuria
	Azotaemia
	Continuous haemodiafiltration
	Dialysis
	Haemodialysis
	Haemofiltration
	Neonatal anuria
	Nephropathy toxic
	Oliguria
	Peritoneal dialysis
	Prerenal failure
	Renal failure
	Renal failure neonatal

	Renal impairment
	Renal impairment neonatal
	Albuminuria
	Blood creatinine abnormal
	Blood creatinine increased
	Crystal nephropathy
	Hypercreatininaemia
	Nephritis
	Protein urine present
	Proteinuria
	Renal function test abnormal
	Urine output decreased

Name of Search	Preferred Term
Modified narrow chronic kidney disease (SMQ)	Artificial kidney device user
	Azotaemia
	Chronic kidney disease
	Coma uraemic
	Diabetic end stage renal disease
	Dialysis
	Dialysis device insertion
	Glomerulonephritis chronic
	Haemodialysis
	Haemofiltration
	Hepatorenal failure
	High turnover osteopathy
	Hyperparathyroidism secondary
	Kidney fibrosis
	Low turnover osteopathy
	Nephrogenic anaemia
	Nephrogenic systemic fibrosis
	Nephrosclerosis
	Oedema due to renal disease
	Pericarditis uraemic
	Peritoneal dialysis
	Renal and liver transplant

	Renal and pancreas transplant
	Renal failure
	Renal osteodystrophy
	Renal replacement therapy
	Renal rickets
	Renal transplant
	Uraemia odour
	Uraemic acidosis
	Uraemic encephalopathy
	Uraemic gastropathy
	Uraemic neuropathy
	Uraemic pruritus
	Uridrosis
	Albumin urine present
	Albuminuria
	Blood creatinine abnormal
	Blood creatinine increased
	Fibrillary glomerulonephritis
	Focal segmental glomerulosclerosis
	Glomerulonephritis
	Glomerulonephritis membranoproliferative
	Glomerulonephritis membranous
	Glomerulonephritis minimal lesion
	Glomerulonephritis proliferative
	Glomerulonephritis rapidly progressive
	Glomerulonephropathy
	Glomerulosclerosis
	Goodpasture's syndrome
	IgA nephropathy
	Immunotactoid glomerulonephritis
	Mesangioproliferative glomerulonephritis
	Microalbuminuria
	Nephritic syndrome
	Nephropathy
	Nephropathy toxic
	Nephrotic syndrome
	Protein urine present

	Proteinuria
	Red blood cells urine positive
	Renal papillary necrosis
	Ultrafiltration failure
	Urinary casts present
	Urine albumin/creatinine ratio abnormal
	Urine albumin/creatinine ratio increased
	Urine output decreased
	Urine protein/creatinine ratio abnormal
	Urine protein/creatinine ratio increased

28: Increased liver function test or Events Involving Hepatic Impairment

Increased liver function test (LFT) or events involving hepatic impairment will be identified using modified hepatocellular damage and hepatitis NEC HLT plus broad Liver related investigations, signs and symptoms SMQ.

Additional preferred terms for modified hepatocellular damage and hepatitis NEC HLT:

Name of Search	Preferred Term
Additional preferred terms for modified hepatocellular damage and hepatitis NEC HLT	Autoimmune hepatitis
	Allergic hepatitis
	Chronic hepatitis
	Drug-induced liver injury
	Hepatitis
	Hepatitis acute
	Hepatitis fulminant
	Hepatitis toxic
	Hepatocellular injury
	Hepatotoxicity
	Liver injury
	Non-alcoholic fatty liver
	Non-alcoholic steatohepatitis
	Nonalcoholic fatty liver disease
	Steatohepatitis

Name of Search	Preferred Term
Liver related investigations, signs and symptoms (SMQ)	Alanine aminotransferase abnormal
	Alanine aminotransferase increased
	Ammonia abnormal
	Ammonia increased
	Ascites
	Aspartate aminotransferase abnormal
	Aspartate aminotransferase increased
	Bacterascites
	Bile output abnormal
	Bile output decreased
	Biliary ascites
	Bilirubin conjugated abnormal
	Bilirubin conjugated increased
	Biopsy liver abnormal
	Blood bilirubin abnormal
	Blood bilirubin increased
	Blood bilirubin unconjugated increased
	Bromosulphthalein test abnormal
	Child-Pugh-Turcotte score increased
	Computerised tomogram liver
	Foetor hepaticus
	Galactose elimination capacity test abnormal
	Galactose elimination capacity test decreased
	Gamma-glutamyltransferase abnormal
	Gamma-glutamyltransferase increased
	Guanase increased
	Hepaplastin abnormal

	Hepaplastin decreased
	Hepatic artery flow decreased
	Hepatic congestion
	Hepatic enzyme abnormal
	Hepatic enzyme decreased
	Hepatic enzyme increased
	Hepatic function abnormal
	Hepatic hydrothorax
	Hepatic hypertrophy
	Hepatic mass
	Hepatic pain
	Hepatic sequestration
	Hepatic vascular resistance increased
	Hepatobiliary scan abnormal
	Hepatomegaly
	Hepatosplenomegaly
	Hyperammonaemia
	Hyperbilirubinaemia
	Hypercholia
	Hypertransaminasaemia
	Kayser-Fleischer ring
	Liver function test abnormal
	Liver function test increased
	Liver induration
	Liver palpable
	Liver scan abnormal
	Liver tenderness
	Mitochondrial aspartate aminotransferase increased
	Molar ratio of total branched-chain amino acid to tyrosine

	Oedema due to hepatic disease
	Perihepatic discomfort
	Retrograde portal vein flow
	Total bile acids increased
	Transaminases abnormal
	Transaminases increased
	Ultrasound liver abnormal
	Urine bilirubin increased
	X-ray hepatobiliary abnormal
	5'nucleotidase increased
	Blood alkaline phosphatase abnormal
	Blood alkaline phosphatase increased
	Blood cholinesterase abnormal
	Blood cholinesterase decreased
	Deficiency of bile secretion
	Glutamate dehydrogenase increased
	Haemorrhagic ascites
	Hepatic fibrosis marker abnormal
	Hepatic fibrosis marker increased
	Hypoalbuminaemia
	Leucine aminopeptidase increased
	Liver iron concentration abnormal
	Liver iron concentration increased
	Periportal oedema
	Peritoneal fluid protein abnormal
	Peritoneal fluid protein decreased
	Peritoneal fluid protein increased
	Pneumobilia
	Portal vein flow decreased

	Portal vein pressure increased
	Retinol binding protein decreased
	Urobilinogen urine decreased
	Urobilinogen urine increased

29: Anemia

Anemia will be identified using following preferred terms:

Name of Search	Preferred Term
Anemia	Anaemia
	Anaemia macrocytic
	Anaemia megloblastic
	Hyperchromic anaemia
	Hypochromic anaemia
	Microcytic anaemia
	Sideroblastic anaemia

30: Thrombocytopenia

Thrombocytopenia will be identified using following preferred terms:

Name of Search	Preferred Term
Thrombocytopenia	Thrombocytopenia
	Immune thrombocytopenic purpura
	Platelet production decreased
	Thrombocytopenic purpura
	Platelet count decreased
	Platelet count abnormal
	Platelet destruction increased
	Platelet disorder
	Platecrit abnormal
	Platecrit decreased

31: Hemolysis

Hemolysis will be identified using broad haemolytic disorders SMQ.

Name of Search	Preferred Term
Hemolysis	ABO haemolytic disease of newborn
	ABO incompatibility
	Acid haemolysin test positive
	Acute haemolytic transfusion reaction
	Alloimmunisation
	Anti A antibody positive
	Anti B antibody positive
	Anti-erythrocyte antibody positive
	Autoimmune haemolytic anaemia
	Blood incompatibility haemolytic anaemia of newborn
	Cardiac haemolytic anaemia
	Cold agglutinins positive
	Cold type haemolytic anaemia
	Coombs direct test positive
	Coombs indirect test positive
	Coombs negative haemolytic anaemia
	Coombs positive haemolytic anaemia
	Coombs test positive
	Delayed haemolytic transfusion reaction
	Erythroblastosis
	Erythroblastosis foetalis
	Evans syndrome
	Extravascular haemolysis
	Glucose-6-phosphate dehydrogenase abnormal
	Glucose-6-phosphate dehydrogenase deficiency
	Haemoglobin urine present
	Haemoglobinaemia
	Haemoglobinuria
	Haemolysis
	Haemolysis neonatal
	Haemolytic anaemia
	Haemolytic transfusion reaction
	Haemolytic uraemic syndrome
	Haemosiderinuria
	Haptoglobin decreased
	Intravascular haemolysis

	Isoimmune haemolytic disease
	Jaundice acholuric
	Microangiopathic haemolytic anaemia
	Paroxysmal nocturnal haemoglobinuria
	Passenger lymphocyte syndrome
	Red cell fragmentation syndrome
	Rhesus antibodies positive
	Rhesus haemolytic disease of newborn
	Rhesus incompatibility
	Transfusion reaction
	Warm type haemolytic anaemia
	Reticulocyte count abnormal
	Reticulocyte count increased
	Reticulocyte percentage abnormal
	Reticulocyte percentage increased
	Reticulocytosis

32: Myalgia

Myalgia will be identified using the following PT: Fibromyalgia, Myalgia, Myalgia intercostal, Myofascial pain syndrome, Neuromuscular pain

33: Serositis

Serositis will be identified using following preferred terms:

Name of Search	Preferred Term
Serositis	Pericardial effusion
	Pericardial rub
	Pericarditis
	Pleural effusion
	Pleural rub
	Pleurisy
	Pleuropericarditis
	Polyserositis
	Serositis
	Peritonitis
	Noninfectious peritonitis
	Ascites

Name of Search	Preferred Term
	Lymphocele
	Chylothorax

34: Potential central nervous system vascular complications

Potential central nervous system (CNS) vascular complications will be identified using ischaemic central nervous system vascular conditions (SMQ).

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