A Phase2A, Randomized, Multicenter, Open-label Pharmacokinetic, and Dose Response Study of Asfotase Alfa in Adult Patients with Pediatric-onset Hypophosphatasia

Unique Protocol ID:	AA-HPP-208
NCT Number:	NCT02797821
EudraCT Number:	2015-003131-35
Date of Statistical Analysis Plan:	28 June 2017

Alexion Pharmaceuticals, Inc.

STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER: **AA-HPP-208**

A PHASE 2A, RANDOMIZED, MULTICENTER, OPEN-LABEL, PHARMACOKINETIC, AND DOSE RESPONSE STUDY OF ASFOTASE ALFA IN ADULT PATIENTS WITH PEDIATRIC-ONSET HYPOPHOSPHATASIA

Author: PPD

Date: June 28, 2017

Version: 2.0 Final

1. APPROVAL SIGNATURES

PPD

28Jun2017

Date dd Mmm yyyy

28 Jun 2017

Date dd Mmm yyyy

29 Jun 2017

Date dd Mmm yyyy

2. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

TABLE OF CONTENTS

LIST OF TABLES

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and acronyms are used in this SAP.

Table 1: Abbreviations and acronyms

Abbreviation or acronym	Explanation
ADA	Anti-drug antibodies
AE	Adverse event
AESI	Adverse events of special interest
ALP	alkaline phosphatase
ANCOVA	analysis of covariance
ATC	anatomic class
CI	Confidence Interval
CS	Clinically significant
FA	Full Analysis
HPP	Hypophosphatasia
IAR	Injection associated reaction
ISR	Injection site reaction
kg	kilogram
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
mmHg	millimeters of mercury
NAb	Neutralizing antibodies
OC	Observed Case
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Per Protocol
PLP	pyridoxal 5′-phosphate
PPi	inorganic pyrophosphate
PT	Preferred Term (MedDRA)
PTAEs	Pre-Treatment Adverse Events
PTH	Parathyroid Hormone
REML	restricted maximum likelihood
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SAS®	Statistical Analysis Software®
SD	standard deviation
SOC	System Organ Class (MedDRA)
TEAEs	Treatment-Emergent Adverse Events
TNSALP	tissue-nonspecific alkaline phosphatase

4. DESCRIPTION OF THE PROTOCOL

The AA-HPP-208 study, "A Phase 2a, Randomized, Multicenter, Open-Label, Pharmacokinetic, and Dose Response Study of Asfotase Alfa in Adult Patients with Pediatric-Onset Hypophosphatasia" is a post-authorization commitment for European Medicines Agency(EMA) to evaluate the pharmacokinetics (PK) and pharmacodynamics (PD) of asfotase alfa following administration of a range of dose regimens that encompasses the dose proven to be effective in children (6.0 milligram [mg]/kilogram[kg]/week) in adult patients with pediatric-onset hypophosphatasia (HPP). A secondary objective is to evaluate the safety and tolerability of asfotase alfa in adult patients with pediatric-onset HPP. The protocol can be referenced for additional details. The schedule of events is attached in Appendix 9.1 of this statistical analysis plan (SAP).

4.1. Changes from Analyses Specified in the Protocol

The primary analysis of the primary endpoint, the change in PPi from Baseline to pre-3rd dose Week 9 has been changed from non-parametric exact Wilcoxon rank-sum test into a restricted maximum likelihood (REML)-based repeated measures mixed model; while non-parametric exact Wilcoxon rank-sum test becomes a supportive analysis.

The Per Protocol Set criteria for study drug compliance has been modified as 100% compliance (i.e. all doses of study drug) is too restrictive. A slightly revised definition will be utilized requiring that patients receive 100% of study drug as planned on Weeks 8 and 9, and at least 80% of study drug overall as specified in the protocol.

All prior and concomitant medications and therapies must be collected from 30 days prior to study entry up until the final study visit. The protocol has inconsistent description of the process for the collection of Concomitant medications and therapies in Table 2, footnote 9 and Section 8.7; this is being clarified with an administrative letter.

4.2. Changes from Analyses Specified in the Previous Version of the Statistical Analysis Plan

This document describes the SAP for final data analyses for the study. The following additions since the previous version 1.0 of the final SAP will apply:

- The primary analysis of the primary endpoint has been changed, as described in Section 4.1. Analysis of covariance (ANCOVA) will be added as sensitivity analyses. A repeated measures mixed model with fixed, categorical effect of visit will be replaced by the same model with the fixed, categorical effect of visit, adjusted for baseline PPi.
- Sensitivity analyses may be employed to exclude biologically implausible outliers in PPi and PLP.
- The following Adverse Events of Special Interest (AESI) will be added for consistency across the asfotase afa development program:
    - Ectopic calcification

  - Lipodystrophy
  - Craniosynostosis
  - Chronic hepatitis

- Analyses on anti-drug antibody (ADA) status and neutralizing antibody (NAb) status at Baseline and post-Baseline will be added. Time to first ADA positive, ADA positive continuously, ADA peak titer, ADA high titer category (>128) and NAb positive will also be summarized.

5. DEFINITIONS

5.1. Efficacy/Pharmacodynamics

5.1.1. Primary Endpoint

The change in inorganic pyrophosphate (PPi) from Baseline to pre-3rd dose Week 9. Baseline is defined as the average of all available pre-dose PPi assessments prior to first SC injection with asfotase alfa (-168, -156, -24, -12h, and pre-dose Day 1). Pre-3rd dose Week 9 is defined as the value obtained prior to the 3rd dose during Week 9.

5.1.2. Secondary Endpoint

The change in pyridoxal 5′-phosphate (PLP) from Baseline to pre-3rd dose Week 9. Baseline is defined as the average of all available pre-dose assessments prior to first SC injection with asfotase alfa (-168, -156, -24, -12h, and pre-dose Day 1). Pre-3rd dose Week 9 is defined as the value obtained prior to the 3rd dose during Week 9.

5.2. Pharmacokinetic Endpoints

Pharmacokinetic endpoints and analysis will be discussed in a separate PK analysis plan.

5.3. Safety

Safety endpoints will include: adverse events (AEs), vital signs, laboratory assessments, physical examination, renal ultrasound, and ophthalmology examination, urine pregnancy tests, and antibodies. Each is described in further detail below.

5.3.1. Adverse Events (AEs)

Adverse events are defined in Protocol Section 9.4.1. This includes serious adverse events [SAEs], injection site reactions [ISRs], injection associated reactions [IARs], discontinuations due to AEs, drug-related serious and severe AEs. Also refer to Appendix 9.3 of this SAP for special handling of AEs.

Adverse events of special interest (AESI) include: ISR, Hypersensitivity (IAR), ectopic calcification, lipodystrophy, craniosynostosis, and chronic hepatitis. The AESI of ISR and hypersensitivity (IAR) will be identified by investigators as captured on case report forms. They will be supplemented by additional events identified by an Alexion clinical review and which are considered to be at least possibly related to study drug. The AESI of ectopic calcification, lipodystrophy, craniosynostosis, and chronic hepatitis will be identified by an Alexion clinical review irrespective of the event relationship to study drug.

5.3.2. Vital Signs

The following vital signs will be recorded at the times specified in Appendix 9.1: systolic and diastolic blood pressure (millimeters of mercury [mmHg]), heart rate (beats/minute), respiratory rate (breaths/minute), and temperature (degrees Celsius [°C] or degrees Fahrenheit [°F]). On dosing days in the clinic or under medical supervision at home, vital signs will be taken within 10 minutes before study drug injection.

5.3.3. Laboratory Assessments

Serum chemistry, hematology, and urinalysis testing will be performed at the times specified in Appendix 9.1. Specific laboratory assessments are provided in Appendix 14.1 of the protocol. Laboratory testing will also include Vitamin D, serum parathyroid hormone (PTH), and urine Ca:Creatinine at the times specified in Appendix 9.1. A central laboratory (Covance) will be used for laboratory testing and Clinical Significance (CS) will be assigned by the investigator.

In addition, a serum sample for tryptase will be collected pre-dose on Day 1 and analyzed if the patient experiences a subsequent Hypersensitivity (IAR). In the case of systemic hypersensitivity reactions, the investigational site will collect laboratory samples for analysis of the following:
• Tryptase within 1 hour from time of reaction onset, if possible (no longer than 2 hours after onset) and at 24 hours (or later) from time of reaction onset
• C5b-9 within 24 hours from time of reaction onset
• Hematology, blood chemistry, and urinalysis within 3 hours from time of reaction onset and at 24 hours from time of reaction onset

Finally, HPP genetic testing will also be performed at entry into the study.

5.3.4. Physical Examinations

At Screening and Baseline visits, a complete physical examination will be performed. Thereafter, limited physical examinations (based on the patient's signs/symptoms) may be performed at the times specified in Appendix 9.1 using a standardized form with a checklist of items: general appearance; skin; head; ear; eye; nose; throat; neck; lymph nodes; chest; heart; abdominal cavity; limbs; central nervous system; and musculoskeletal. All physical examinations (complete and limited) will include weight (using a calibrated scale) and examination of asfotase alfa injection sites for potential reaction(s). The Screening and Baseline physical examination will include height (using a wall-mounted standiometer).

5.3.5. Renal Ultrasound

A renal ultrasound will be performed at the times specified in Appendix 9.1 to assess for the presence of nephrocalcinosis.

5.3.6. Ophthalmology Assessment

A full ophthalmology examination will be performed at the times specified in Appendix 9.1. The examination will assess for papilledema and signs of ectopic calcification, and will include assessments of visual acuity; adnexa; and slit-lamp biomicroscopy with examination of anterior chamber, lens, conjunctiva, cornea, fundus, and other.

5.3.7. Urine Pregnancy Test

Pregnancy data will be performed in all female patients of childbearing potential.

5.3.8. Anti-Drug Antibodies (ADA) and Neutralizing Antibodies (NAb)

Patients will be monitored for development of antibody production against asfotase alfa (asfotase alfa ADA) throughout this study at the times specified in Appendix 9.1. Serum will be collected and analyzed for the presence of ADA, and if positive, further analyzed for titer and NAb.

The following definitions will be applied to categorize ADA and NAb post-baseline results:

- ADA positive status
  - Positive (Ever)

    Patients who had at least 1 positive post-baseline value for ADA
  - Negative (Always)

    Patients who never had a positive post-baseline value for ADA
- ADA continuously positive status (evaluated in the exposure based analysis only)
  - Continuously Positive Criteria:

    To be continuously positive a patient must have achieved, and sustained, uninterrupted positive results for ADA during and through the end of their series of post-baseline ADA measures. At a minimum, patients must be positive for the last 2 post-baseline measures of all time points when patients were tested for ADAs (e.g., being positive only at the last measure is not sufficient to confer continuously positive status).

    Patients whose ADA Status was "Positive (Ever)" and had ADA values that met the Continuously Positive Criteria described above.
  - Not Continuously Positive

    Patients whose ADA Status was "Negative (Always)", or

    Patients whose ADA Status was "Positive (Ever)" and had ADA values that did not met the Continuously Positive Criteria described above.
- ADA high and low titer status (evaluated in the exposure based analysis only)
  - High Titer

    Patients whose ADA Status was "Positive (Ever)" and whose maximum post-baseline value for ADA was > 128.
  - Low Titer

    Patients whose ADA Status was "Positive (Ever)" and whose maximum post-baseline value for ADA was <= 128.
- NAb positive status
  - NAb Positive Criteria:

    Neutralizing antibody subgroups will be determined using the NAb percent inhibition levels where < 4.478 will be considered "Negative" and >=4.478 will be considered "Positive".

    NAb positive status may only be determined for observations that were concurrently positive for ADA. Accordingly, all observations where patients were negative for ADAs will automatically set the NAb status to negative and any analyses of NAb inhibition will also be omitted.

- Positive (Ever)

  Patients who had at least 1 positive post-baseline value for NAb

- Negative (Always)

  Patients who never had a positive post-baseline value for NAb

6. DATA SETS ANALYZED (STUDY POPULATIONS)

6.1. Full Analysis (FA) Set

Patients who have been randomized and received at least one dose of study drug that have at least one pre-treatment and one on-treatment PPi result will be included in the FA set. Patients will be included in the analyses according to the treatment cohort to which they were randomized, irrespective of the treatment they actually received.

6.2. Per Protocol (PP) Set

Patients meeting the definition of FA Set (see above) and who are without major protocol deviations will be included in the PP Set. Major protocol deviations will include:

- Non-compliance with study treatment; compliance for a single dose will be defined as receiving the assigned cohort dose with a 10% window around expected mg administered.
- Not receiving all doses of study drug during Weeks 8 and 9 and at least 80% of study drug as specified in the protocol.
- Not receiving the correct study treatment
- Failing to meet key (pre-defined) eligibility criteria
- Other major protocol violations

Determination of whether or not a patient will be excluded from the PP Set will be made prior to the database lock.

6.3. Safety Set

All patients who received at least one dose of study drug will be included in the Safety Set. Patients will be considered, for safety analysis, to be in the treatment cohort of the treatment they actually received.

6.4. PK Analysis Set

The PK Analysis Set will be described in a separate PK analysis plan.

7. STATISTICAL ANALYSIS

For the statistical analyses below, descriptive statistics (n, mean, median, standard deviation [SD], 95% confidence interval [CI], minimum, and maximum) will be provided for each continuous variable, and frequencies and percentages will be provided for each categorical variable. Analyses will be conducted using Statistical Analysis Software (SAS©) version 9.2 or higher. Any hypothesis testing described will be 2-sided and performed at the 0.05 level of significance; p-values <0.05 will be considered statistically significant. In general, analyses will be presented by treatment cohort, and overall where specified. The 3 treatment cohorts are listed below:

Cohort #	Description	Label in output
Cohort 1:	Asfotase alfa 0.5 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 0.5 mg/kg 3 times per week	0.5 mg/kg cohort
Cohort 2:	Asfotase alfa 2.0 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 2.0 mg/kg 3 times per week	2.0 mg/kg cohort
Cohort 3:	Asfotase alfa 3.0 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 3.0 mg/kg 3 times per week	3.0 mg/kg cohort

7.1. Study Patients

7.1.1. Disposition of Patients

The number and percent of patients completing the study will be described. For patients who discontinued the study, the reason for discontinuation will be summarized. In addition, the number of patients who discontinued study drug permanently due to an AE will be summarized. Information will be reported using all randomized patients and summary statistics will be presented by treatment cohort and overall.

A listing of patients indicating randomization to treatment, attendance at each visit (home or in-clinic), discontinuation from the study, and completion of the study will also be generated.

7.1.2. Protocol Deviations

The number and percent of patients with specific protocol deviations will be summarized using all randomized patients. The following protocol deviations will be checked programmatically from the database:

1. Patients who violated any inclusion/exclusion criteria.
2. Patients who missed one of the scheduled doses of study drug.
3. Patients who did not receive the correct study treatment.

4. Patients who received doses outside the 10% window for expected mg dosage administration.

Protocol deviations from monitoring reports and other relevant sources will also be reviewed, and any important deviations will be included in the list that is summarized and reported.

Summary statistics will be presented by treatment cohort and overall.

7.1.3. Demographics, Disease Characteristics, and History

All demographic, baseline characteristic, and history information will be summarized using the FA Set by treatment cohort and overall. No formal hypothesis testing will be performed to compare differences between treatment cohorts.

Summary statistics will be presented using descriptive statistics (n, mean, SD, 95% CI, median, minimum, and maximum) or frequencies and percentages, as appropriate.

7.1.3.1. Demographics

The following demographic variables will be summarized:

- Sex
- Ethnicity
- Race
- Japanese descent (yes/no)
- Age (years) at Enrollment
- Age (years) at First Injection

7.1.3.2. Disease Characteristics

The following disease characteristics will be summarized:

- Age at First Sign/Symptom (years)
- Age at Diagnosis (years)
- Time since First Sign/Symptom (see Appendix 9.3)
- Time since Diagnosis (see Appendix 9.3)
- TNSALP Gene Mutation
  - Method of Sequencing (whole exome/gene/targeted mutation/targeted next generation/panel/other/unknown)
  - Polymorphism present (yes/no)
  - Polymorphism status (homozygous/heterozygous)
- Documented history (yes/no) of:
  - serum alkaline phosphatase (ALP) levels below the age-adjusted normal range
  - tissue-nonspecific alkaline phosphatase (TNSALP) gene mutation(s)

- documented history of PLP above the upper limit of normal at Screening

All disease characteristics, including the TNSALP gene mutation reported, will be presented by treatment cohort and patient identification in data listings.

7.1.3.3. Medical History and Baseline Physical Examination

The number and percentage of patients with medical history findings will be summarized for the FA Set by treatment cohort and overall by The Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT). Additionally, HPP-specific medical history will be summarized, including a summary of first known HPP symptoms. Lastly, the number of permanent teeth lost and number of episodes of pneumonia will be summarized.

For the physical examination, the number and percentage of patients with abnormal findings at Baseline for each body system will be summarized by treatment cohort and overall.

All medical history and baseline physical examination data will be presented in by-patient data listings, including the medical history verbatim text describing each history event or first known symptom and any abnormal physical examination free text findings. Height at study entry and postmenopausal status for females at study entry will also be presented in listings.

7.1.4. Prior and Concomitant Medications / Therapies

Concomitant medication/therapy data and prior- medication data will be presented in by-patient data listings. See Appendix 9.1 for additional details on the definition of prior and concomitant medications/therapies and rules for handling missing or incomplete start and stop dates. Concomitant medications and therapies will also be summarized in tabular form by anatomic class (ATC) level 3 and generic name for the FA Set by treatment cohort and overall using frequencies and percentages. The WHO Drug dictionary (DD) version from MAR2015 or later will be used to code the medications.

7.2. Efficacy/Pharmacodynamic Analyses

The analyses and summaries will be based on the FA Set.

7.2.1. Primary Endpoint

The primary endpoint for this study is the change in PPi from Baseline to pre-3rd dose Week 9. Change from Baseline in PPi will be the primary variable. Primary analysis will be performed by using a restricted maximum likelihood (REML)-based repeated measures mixed model, fit for each treatment cohort to estimate the change at each pre-dose (trough) timepoint starting at Week 4 (Day 22) through Week 9 (Day 61). The primary hypotheses will test whether the differences between the cohorts differ from zero at pre-3rd dose Week 9. The Week 3 (Day 15) pre-dose timepoint will not be included in the model assessing change since this is expected to be similar to a baseline level of PPi after the first non-dosing period. The analysis will include the fixed, categorical effect of visit, adjusted for baseline PPi, gender, baseline weight group ($\geq$ median versus < median), and study drug lot assignment. An unstructured covariance structure will be used to model the within-patient errors. If this analysis fails to converge, the following structures will be tested and the final covariance structure will be determined by Akaike's information criterion (AIC): first-order autoregressive, compound symmetry, and Toeplitz. The Kenward-

Roger approximation will be used to estimate denominator degrees of freedom. The estimated mean change from Baseline at each visit from the model will be provided, along with 95% CIs and p-values.

A fixed sequence testing procedure will be performed with the comparison of the 3.0 mg/kg cohort compared with the 0.5 mg/kg cohort being performed first, and the hypothesis testing for the second comparison 2.0 mg/kg cohort compared with the 0.5 mg/kg cohort only being performed if the null hypothesis is rejected for the previous comparison at a significance level of 0.05 (p-value <0.05). The primary endpoint will be met if the null hypothesis is rejected for both comparisons at a significance level of 0.05 (both p-values <0.05).

The same model will additionally be employed with the fixed, categorical effect of visit, adjusted for baseline PPi.

A similar modeling approach will be employed to explore the effect of treatment cohort on the change from Baseline in PPi at each pre-dose timepoint.

As supportive analysis, the non-parametric exact Wilcoxon rank-sum test will be used to assess differences between treatment cohorts, using both last observation carried forward (LOCF) and observed case (OC) methods. The location shift in the change from Baseline distributions and 95% CI for the above comparisons will be reported using the Hodges-Lehmann-Sen estimate and the exact confidence limits reported.

To examine the change from Baseline in PPi, descriptive statistics for the absolute PPi level and the change from Baseline will be summarized at all study timepoints by treatment cohort, spanning from Week 1 to Week 13; Baseline will be calculated in the same way as for the primary analysis. Within cohort, the change from Baseline to all study timepoints will be analyzed using the non-parametric Wilcoxon signed-rank test.

Individual and mean PPi levels for each cohort versus time curves will be presented with normal references ranges indicated (see Appendix 9.3). Please refer to Table 4 on how to handle the values fall under the lower limit of quantification (LLOQ). Additional analysis of PPi data may be performed if considered useful.

The above analyses for PPi will additionally be performed using the PP Set.

7.2.1.1. Handling of Dropouts or Missing Data

The protocol schedule requires 5 pre-treatment baseline PPi samples (-168, -156, -24, -12h, and pre-dose Day 1) be collected. The average value will be used for calculating Baseline PPi; therefore, at least 1 pretreatment baseline observation is required for the patient to be included for the assessment of the primary analysis. Patients will also be required to have at least 1 on-treatment PPi observation to be included in the FA Set. For the analyses of changes from Baseline using repeated measures mixed modeling, missing values will not be imputed based on a Missing at Random Assumption (MAR). Missing values at the pre-3rd dose Week 9 assessment will be imputed with the last available on-treatment pre-dose trough assessment prior to pre-3rd dose Week 9 using LOCF. This imputation will occur where indicated.

7.2.1.2. Subgroup Analysis

The randomization for this study is planned to be stratified by gender; therefore, all descriptive analyses discussed in Section 7.2.1 will additionally be performed stratified by gender.

7.2.1.3. Multicenter Studies

Up to 4 centers are expected for this study and the number of patients at each center by cohort is not expected to be large enough to produce meaningful summaries by center; therefore analyses by center will not be performed.

7.2.1.4. Hypothesis Testing and Significance Level

A two-sided Type I error rate of 0.05 will be used to define statistical significance. The primary endpoint will be met if the null hypothesis is rejected for both comparisons described in Section 7.2.1 at a significance level of 0.05, i.e. both p-values <0.05. For the primary endpoint, a fixed sequence testing procedure will be used to control the Type I error rate. Statistical adjustment of p-values will not be performed.

7.2.1.5. Sensitivity Analyses

The change in PPi from Baseline to pre-3rd dose Week 9 will also be analyzed using analysis of covariance (ANCOVA), with treatment as fixed factor, baseline PPi, gender, baseline weight group (≥ median versus < median), and study drug lot assignment as covariates, and using both LOCF and OC methods in the FA Set.

The tests will compare 2.0 mg/kg cohort to 0.5 mg/kg cohort, and 3.0 mg/kg cohort to 0.5 mg/kg cohort. Ninety-five percent CIs will be presented together with the estimated p-values.

In addition, sensitivity analyses will be performed if any medications are administered to the patient that might impact the results used in the primary analysis of PPi.

Sensitivity analyses may be employed that include only the patients that receive 100% of the study drug doses through Week 9, as specified in the protocol.

Finally, sensitivity analyses may be employed to exclude biologically implausible outliers in PPi and PLP.

7.2.2. Secondary Endpoint

For the secondary endpoint, PLP change from Baseline to pre-3rd dose Week 9, similar methods as for PPi will be employed in the FA Set and PP Set.

Sensitivity analyses for PLP may be employed for any patients without taking any vitamin B6 or with consistent input of vitamin B6, defined as taking same dose of vitamin B6 during the study. Vitamin B6 usage will be obtained from the concomitant medications.

Additionally for sensitivity, the within-patient mean absolute levels of the pre-treatment "fasting" PLP samples (-168 h, -24 h, pre-dose Day 1) and the "non-fasting" PLP samples (-156 h, -12 h) will be compared using the exact Wilcoxon rank-sum test for all patients in the FA Set. If a statistical difference is detected between the pre-treatment "fasting" and "non-fasting" samples, given samples on treatment are collected under both "fasting" and "non-fasting"

conditions, the change to each post-treatment timepoint will be evaluated descriptively using the matched "fasting" or "non-fasting" average at Baseline; accordingly, "fasting" post-treatment samples will use a "fasting" Baseline and "non-fasting" post-treatment samples will use a "non-fasting" Baseline.

Please refer to Table 4 on how to handle the values fall under the lower limit of quantification (LLOQ).

7.2.3. Tertiary Analyses

Not Applicable.

7.2.4. Other Efficacy Analyses

Not Applicable.

7.2.5. Pharmacokinetic and Pharmacodynamic Analyses

Pharamcokinetic analyses and analyses relating PK and PD will be described in a separate analysis plan.

7.3. Safety Analyses

All safety analyses will be conducted on the Safety Set based on the treatment actually received. All safety data will be summarized in tabular format by treatment cohort and overall, and all data will be in by-patient data listings. See Appendix 9.3 for the definition of Treatment Baseline for Safety analyses. No formal hypothesis testing is planned. Missing or invalid safety data will not be replaced.

7.3.1. Study Duration, Treatment Compliance, and Exposure

Study duration, treatment compliance, and exposure will be summarized for the Safety Set by treatment cohort, according to the treatment actually received.

Study duration is defined as the time from first dose to the end of the main study period (ie, Week 13: Day 87) or study discontinuation date, whichever occurs first. The period after Day 87 up to the Safety Follow-up call at 90 days after last dose will not be included in the calculation.

Study treatment compliance will be defined as the percentage of reported injections taken out of the total number of expected reported injections from the dosing diaries up to the point of study completion or discontinuation. If a patient discontinues prematurely from the study, his or her compliance will be based on the period up to the point of discontinuation from the study. A separate study treatment compliance analysis will be conducted counting only reported injections where the milligram administered complies within a 10% window of the expected dose based on the weight linked to the dosing event.

Study drug administration data will be presented by treatment cohort in a listing. The date/time of first injection, end time of last injection (if multiple), injection location(s), total volume administered (mL), and number of injections will be included. In addition, tabular summaries will be produced by treatment cohort for the average daily dose (mg/kg) including all expected

dosing events on study and average weekly dose (mg/kg/week) during the multiple Dosing Period, derived from the volume administered and weight linked to each dosing event.

7.3.2. Adverse Events (AEs)

Adverse events will be coded in MedDRA and presented by SOC and PT. Adverse events occurring on or after first treatment, treatment-emergent AEs (TEAEs), will be tabulated and presented by treatment cohort and overall. Summaries for pre-treatment AEs (PTAEs) will be included only where appropriate and specified. See Appendix 9.3 of the SAP for further details on the difference between TEAEs and PTAEs. Study displays to be included are described below.

7.3.2.1. Overall Summary of Adverse Events

An overall summary of the following categories of AEs will be presented by treatment cohort and overall, including summaries of events (n) and number of patients with events (n, %): TEAEs, treatment-emergent SAEs, and treatment-emergent non-SAEs. Within each category, the following subcategories will also be summarized:

- Mild/Moderate/Severe TEAEs
- Related TEAEs (possibly, probably, definitely related, or missing relationship)
- TEAEs leading to withdrawal from the study
- TEAEs leading to death
- AESI, see Section 7.3.2.7

A summary of events (n) and number of patients with events (n, %) for PTAEs will also be included with the following subcategories: Mild/Moderate/Severe AEs, AEs leading to withdrawal, and AEs leading to death (note that Related AEs and AESI do not apply to PTAEs).

A listing of all TEAEs by treatment cohort and patient will be presented. Separate listings will be produced for treatment-emergent SAEs, AEs leading to study drug withdrawal, AEs resulting in death, and PTAEs.

7.3.2.2. AEs by SOC and PT

The number of TEAEs and the number and percentage of patients with events will be presented by SOC and PT. Patients are counted once in each SOC and PT. Percentages will be based on the total number of treated patients in the treatment cohort. Treatment-emergent SAEs, treatment-emergent non-SAEs, and PTAEs will be summarized using the same approach.

7.3.2.3. AEs by PT

The number of TEAEs and the number and percentage of patients with events will be presented by PT. Patients are counted once in each PT. Percentages will be based on the total number of treated patients in the treatment cohort.

7.3.2.4. AEs and SAEs by SOC, PT, and Relationship

The number and percentage of patients with events will be presented by SOC and PT by grouped relationship (related, not related). If a patient has more than one occurrence of an AE, the strongest relationship to study treatment will be used in the summary table.

In addition, the number of AEs and the number and percentage of patients with AEs will be summarized by SOC and PT, and relationship result: not related, unlikely related, possibly related, probably related, or definitely related.

Lastly, the number of Related TEAEs and the number and percentage of patients with Related TEAEs will be summarized by SOC and PT, and separately by PT. The same analysis will be produced for Related treatment-emergent SAEs.

7.3.2.5. AEs by SOC, PT, and Severity

The number and percentage of patients with events will be presented by SOC and PT by severity (mild, moderate, severe). If a patient has more than one occurrence of an AE, the most severe occurrence will be used in the summary table.

7.3.2.6. Deaths

The AEs resulting in death will be presented in a listing.

7.3.2.7. Adverse Events of Special Interest

7.3.2.7.1. Hypersensitivity (IARs) and Injection Site Reactions (ISRs)

The number of Hypersensitivity (IARs) and number and percentage of patients with Hypersensitivity (IARs) will be presented by treatment cohort and overall by SOC and PT; the same analysis will be repeated separately for ISRs. Separate listings of Hypersensitivity (IARs) and ISRs will be produced.

7.3.2.7.2. Adverse Events of Special Interest: Ectopic Calcification

All observed MedDRA PTs identified in the Alexion clinical review to be ectopic calcification AESIs will be presented alphabetically in a data listing.

The number of ectopic calcification AESIs, TEAEs within this AESI category, and the number and percentage of patients with events will be presented by treatment cohort and overall by SOC and PT.

7.3.2.7.3. Adverse Events of Special Interest: Lipodystrophy

Events of lipodystrophy will be analyzed using the same approach described above for events of ectopic calcification.

7.3.2.7.4. Adverse Events of Special Interest: Craniosynostosis

Events of craniosynostosis will be analyzed using the same approach described above for events of ectopic calcification.

7.3.2.7.5. Adverse Events of Special Interest: Chronic Hepatitis

Events of chronic hepatitis will be analyzed using the same approach described above for events of ectopic calcification.

7.3.3. Other Safety

7.3.3.1. Analyses for Laboratory Tests

Actual values and changes from treatment baseline will be summarized descriptively for patients with available data for each laboratory parameter by treatment cohort and overall. Missing laboratory data will not be imputed and only scheduled assessments will be summarized in table summaries; any unscheduled assessments will be included in by-patient data listings and will be flagged as such. A summary for "Last Assessment" will be included for the last available post-treatment baseline result for each patient. See Appendix 9.3 for the definition of Treatment Baseline for Safety analyses, and refer to Table 4 on how to handle the PTH values fall under the LLOQ.

All laboratory values will be classified as normal, below normal (low), or above normal (high) based on normal ranges supplied by the central laboratory and shift tables will be produced showing the shift from treatment baseline to post-treatment baseline assessments. Frequencies of abnormal values will be presented in tabular form. A summary of shifts to Last Assessment will also be included.

For selected laboratory assessments, for example, ALP, figures over time may be produced. For purposes of analyses, laboratory results based upon standardized units will be used.

A listing of all laboratory data will be presented by treatment cohort, patient identification, visit, and laboratory parameter.

7.3.3.2. Vital Signs

Actual values and changes from treatment baseline in vital signs (blood pressure, heart rate, respiratory rate, temperature) at each visit will be summarized descriptively for patients with available data by treatment cohort and overall. Missing vital signs data will not be imputed and only scheduled assessments will be summarized in tables; unscheduled assessments will be presented in by-patient data listings and flagged as such. A summary for "Last Assessment" will be included for the last available post-treatment baseline result for each patient.

A listing of all vital signs data will be presented by treatment cohort, patient identification, and visit with each vital sign result for the visit listed in the same row. A separate listing of patient weight data at each visit will also be presented with unit standardized to kg.

7.3.3.3. Physical Examination

Physical examination findings will be summarized descriptively by treatment cohort and overall for each timepoint using counts and percentages. A summary for "Last Assessment" will be included for the last available post-treatment baseline result for each patient. A listing of all physical examination data will be presented by treatment cohort, patient identification, visit, and body system.

7.3.3.4. Renal Ultrasound

The following renal ultrasound findings will be summarized by treatment cohort and overall for each timepoint using counts and percentages:

-Nephrocalcinosis (yes/no)

-Normal/Abnormal Not Clinically Significant not related to underlying condition/Abnormal Not Clinically Significant related to underlying condition/Abnormal Clinically Significant

Listings of all renal ultrasound results will be provided by treatment cohort, patient identification, and visit, including any free text response for 'Findings'.

7.3.3.5. Ophthalmology Assessment

The following ophthalmology examination findings will be summarized by treatment cohort and overall for each timepoint and eye (left and right), using counts and percentages:

Normal/Abnormal Not Clinically Significant not related to underlying condition/Abnormal Not Clinically Significant related to underlying condition/Abnormal Clinically Significant for the following:

- Adnexa, Eye Movements, Intraocular Pressure
- Visual Acuity
- Slit-lamp Biomicroscopy categories: Anterior Chamber, Lens, Conjunctiva, Cornea, Fundus, Other

Is Visual Acuity impacted by any abnormality observed' (yes/no)

Listings of all ophthalmology assessments will be provided by treatment cohort, patient identification, and visit, including any free text response for 'Findings' where calcifications should be noted.

7.3.3.6. Pregnancy

Pregnancy data will be provided in a listing by treatment cohort and patient identification.

7.3.3.7. Anti-Drug Antibodies and Neutralizing Antibodies

The following frequency counts and/or percentages will be provided for ADA:

- Frequency counts of patients who were missing all ADA values at Baseline, had ADA values at Baseline, missing all ADA values post-baseline, had ADA values post-baseline, or had ADA values present both at Baseline and post-baseline.
- Frequency counts and percentages of patients who were "ADA Negative" or "ADA Positive" at Baseline. The denominator will be the number of patients who had ADA values reported at Baseline.
- Frequency counts and percentages of patients who were:
    - "Always ADA Negative"
    - "Ever ADA Positive"
    - "ADA Positive Continuously"
    - "ADA High Titer Category (>128)"

The denominator will be the number of patients who had ADA values reported post-baseline.

- Frequency counts and percentages of patients who were "ADA Negative" or "ADA Positive" at Baseline (in patients who had ADA values reported at both Baseline and post-baseline). The denominator will be the number of patients who had ADA values reported both at Baseline and post-baseline.
- Frequency counts and percentages of patients that had ADA values reported who were:
    - "Always ADA Negative"
    - "Ever ADA Positive"
    - "ADA Positive Continuously"
    - "ADA High Titer Category (>128)"

  The denominator will be the number of patients who had ADA values reported at both Baseline and post-baseline.
- Frequency counts and percentages for shifts in ADA status from:
    - "ADA Negative" to "Always ADA Negative"
    - "ADA Negative" to "Ever ADA Positive"
    - "ADA Positive" to "Always ADA Negative"
    - "ADA Positive" to "Ever ADA Positive"
    - "ADA Negative" to "ADA Positive Continuously"
    - "ADA Positive" to "ADA High Titer Category (>128)"

  The denominator will be the number of patients who had ADA Status reported at both Baseline and post-baseline.

The following frequency counts and/or percentages will be provided for NAb:

- Frequency counts of patients who were "ADA Positive" at baseline or "Ever ADA Positive" at post-baseline. This summary will identify the population of patients for relevant assessment of NAb.
    - Frequency counts of patients who were missing all NAb values at Baseline, or had NAb values at Baseline, and patients who were "ADA Positive" at baseline.
    - Frequency counts and percentages for "NAb Negative" and "NAb Positive" at Baseline in patients who were "ADA Positive" at Baseline. The denominator will be the number of patients who had NAb values at Baseline and were "ADA Positive" at Baseline.
    - Frequency counts of patients who were missing all NAb values at post-baseline, or had NAb values at post-baseline, and patients who were "Ever ADA Positive" post-baseline.
    - Frequency counts and percentages for "Always NAb Negative" and "Ever NAb Positive" post-baseline in patients who were "Ever ADA Positive" post-baseline. The denominator will be the number of patients who had NAb values post-baseline and were "Ever ADA Positive" post-baseline.

The number and percentage of patients that do and do not develop ADA to asfotase alfa at each time point will also be presented. Additionally, descriptive statistics for ADA titers at each time point will be presented, including counts and percentages for each titer, and median, minimum, and peak titer. For those patients that are ADA positive, the number and percentage of patients

with NAb positive and negative status will be presented along with continuous descriptive statistics for the NAb % inhibition for NAb positive patients.

The peak ADA titer and last ADA titer will be summarized with descriptive statistics, as well as the peak NAb and last NAb % inhibition in the patients that are both ADA and NAb positive overall. The peak and last NAb % inhibition will be evaluated only in results that have both ADA and NAb positive status.

The following will be summarized descriptively: the time to first post-baseline ADA positive, the time to first post-baseline ADA positive continuously, the time to first ADA peak titer, the time to first ADA high titer category (>128), the time to first NAb positive, and the time to first NAb peak % inhibition. Finally, graphical displays will be presented showing titer levels over time, with an overlay of PPi and (separately) PLP levels over time. Listings of all antibody data will be produced by treatment cohort, patient identification, and visit.

Graphical displays will be presented as appropriate.

8. REFERENCES

1. Charles River Final Report, Charles River Laboratories Preclinical Services, Test Facility Study No. 315304, Quantitative Determination of Pyrophosphate (PPi) Concentration in Normal Human Plasma Samples Using a PPi Assay (Normal Reference Range). Report Date: 18 September 2013.

2. Biotrial Analytical Report, Biotrial Bioanalytical Services Inc. study number: P11137_CZR, Determination of Pyridoxal-5-phosphate in Human Plasma by LC/MS/MS. Report Date: 09-OCT-2013.

9. APPENDICES

9.1. Protocol Schedules of Events

Table 2: Protocol Schedule of events

Assessment	Screening	Run-in Period	Main Study Period								Safety F/U Call[1]
			Single Dose	No Dosing	Weekly Dosing (3x/week)				No Dosing		
Study Day[2,3] Study Week[2,3]	W -12 to W -2	D -7 to D -1 (W -1)	D1-D7 (W1)	D8-D14 (W2)	D15-D35 (W3-W5)	D36-D42 (W6)	D43-D56 (W7-W8)	D57-D63 (W9)	D64-D80 (W10-W12)	D81-D87 (W13)	EOS+ 3m
Informed Consent[4]	X										
Inclusion/ Exclusion Criteria	X										
Demographics[5]	X										
Medical history[6]	X										
HPP gene mutation analysis[7]		X									
Concomitant medications[8]	X	X	X	X	X	X	X	X	X	X	
Concomitant therapies[8]	X	X	X	X	X	X	X	X	X	X	
Physical examinations[9]	X	X	X					X		X	
Vital signs[10]	X	X	X	X	X		X	X	X	X	
Body Weight[9]	X	X	X	X	X	X	X	X	X	X	
Randomization		X									
In-Clinic[11]	X	X	X					X		X	
Study drug administration-Single Dose[12,13]			X								
Study drug administration-3x Weekly Dosing[12,13]					X	X	X	X			
Chemistry, hematology, urinalysis[14]	X		X					X		X	

Assessment	Screening	Run-in Period	Main Study Period								Safety F/U Call[1]
			Single Dose	No Dosing	Weekly Dosing (3x/week)				No Dosing		
Study Day[2,3] Study Week[2,3]	W -12 to W -2	D -7 to D -1 (W -1)	D1-D7 (W1)	D8-D14 (W2)	D15-D35 (W3-W5)	D36-D42 (W6)	D43-D56 (W7-W8)	D57-D63 (W9)	D64-D80 (W10-W12)	D81-D87 (W13)	EOS+ 3m
Lab Tests (vitamin D, serum PTH, urine Ca:creatinine)[14]	X		X					X		X	
Pregnancy Test	X	X	X					X		X	
Renal ultrasound		X								X	
Eye exam[15]		X								X	
Pharmaco-dynamics (PPi and PLP)[16,17]	X	X	X	X	X		X	X	X	X	
Anti-drug antibodies[16,17]			X	X	X		X	X	X	X	
Pharmacokinetics[16,17]			X	X	X		X	X	X	X	
Adverse events[18]		X – *Continuous Monitoring*									

Abbreviations: EOS = end-of-study; F/U = follow-up; AE = adverse event; HR = heart rate; IAR = injection-associated reaction; PK = pharmacokinetics; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate; PTH = parathyroid hormone; m = month; W = Week; d = day.

NOTE: Patients may be asked to provide additional blood samples at an unscheduled visit at the request of the Sponsor or Investigator as part of a safety evaluation or for adjustment of the drug dose.

1 The Safety Follow-Up Call will occur 3 months after the last dose of study drug. Males and Females of child-bearing potential will be followed up for birth control and pregnancy information. Any reported pregnancies in female patients or female partners of male patients will be followed until the outcome of the pregnancy is known. Refer to Section 9.4.1.10 for further details.

2 The Week 1, Day 1 single dose must be administered on a Monday, and the multiple dosing must be administered on Mondays, Wednesdays, and Fridays during the weeks indicated.

3 To facilitate scheduling of required study assessments at each visit, the study visit may be shortened or prolonged, as necessary, as long as the specified order of assessments and/or availability of results pertaining to laboratory work (including PK and pregnancy and asfotase alfa anti-drug antibody testing) and vital signs are adhered to.

4 If the time period between the initial Screening and the re-Screening is >12 weeks, all Screening procedures must be repeated.

5 Demographics will be collected as permitted by region and may include Date and region of birth, age, sex, ethnicity, race, and whether the patient is of Japanese descent.

6 Medical history includes general medical history and HPP-specific medical history from historical medical records (eg, X-rays, videos, hospitalization records). Details of loss of adult teeth will be included as part of medical history.

7 HPP gene mutation analysis will be performed at Baseline if results (from within 49 days of Baseline) are not available in the medical records.

8 Concomitant medications and therapies must be collected from 30 days prior to study entry up until the final study visit. All available historical use of certain medications (ie, related to HPP, growth, etc.) will be captured.

9 Physical examination will include assessment of general appearance; skin; head, ear, eye, nose, and throat; neck; lymph nodes; chest; heart; abdominal cavity; limbs; central nervous system; musculoskeletal. At Screening and Baseline visits, a complete physical examination will be performed. Thereafter, limited physical examinations (based on the patient's signs/symptoms) may be performed. All physical examinations (complete and limited) will include weight (using a calibrated scale) and examination of asfotase alfa injection sites for potential reaction(s). The Screening and Baseline physical examination will include height (using a wall-mounted standiometer).

[10] Vital signs include blood pressure, heart rate, respiratory rate, and temperature. On dosing days in the clinic or under medical supervision at home, vital signs will be taken within 10 minutes before study drug injection.
[11] In-clinic or inpatient study visits will occur during Screening, the Run-In Period (Week -1), Week 1, Week 9, and Week 13.
[12] Urine and serum pregnancy testing is required for women of childbearing potential only. Results of urine pregnancy testing must be negative prior to IP administration (when applicable).
[13] Study drug will be administered in the clinic for the first dose and all other doses that occur during study visits. All other study drug administration will occur at home under the supervision of home healthcare.
[14] All patients are required to fast for 8 hours prior to laboratory testing (water all ad libitum). All urine and blood samples for laboratory assessments must be collected prior to study drug administration. Single exception is blood sample collection for analysis of PK parameters at required post-dose timepoints. For all patients, fasting samples should be collected at the beginning of the visit day, before any other study-related procedures are performed.
[15] A full ophthalmology examination will be performed at scheduled visits. The examination will assess for papilledema and signs of ectopic calcification, and will include assessments of visual acuity; adnexa; and slit-lamp biomicroscopy with examination of anterior chamber, lens, conjunctiva, cornea, and fundus. The ophthalmology examination should be performed by a qualified ophthalmologist. Sites will be provided ophthalmologic worksheets required to complete the full exam. Ophthalmology exams may be performed by a qualified optometrist (i.e., Doctor of Optometry, O.D.) as long as the optometrist works under the supervision of an ophthalmologist.
[16] Asfotase alfa anti-drug antibody testing and PK/PD analyses must be performed on samples obtained at the same time (ie, pre-dose samples from the same study visit).
[17] Refer to Table 3 for timing of PK/PD and asfotase alfa anti-drug antibody assessments.
[18] For acute or severe IARs (eg, with signs/symptoms of hypersensitivity, irrespective of the time from administration of study drug to onset), additional blood and urine samples must be collected to assess the reaction. Refer to Section 9.5 for details of sample collection.

Table 3: Protocol schedule of events for Pharmacokinetic, Pharmacodynamic, and Immunogenicity Assessments

	Week	Sampling Times	Dosing[2]	PK, PPi and PLP	ADA
Run-in Period	Week -1 (establish baseline)	Day -7: -168h, -156h pre-dose Day -1: -24h, -12h pre-dose	NA	PPi and PLP only	NA
Single Dose	Week 1	Day 1: pre-dose; post-dose 6h, 12h	D1 (single-dose)	All	Day 1: pre-dose
No Dosing	Week 1	Day 2: post-D1 dose 24h, 32h, 36h Day 3: post-D1 dose 48h, 56h, 60h Day 4: post-D1 dose 72h Day 5: post-D1 dose 96h Day 6: post-D1 dose 120h Day 7: post-D1 dose 144h	None	All	
No Dosing	Week 2	Day 8: post-D1 dose 168h Day 11: post-D1 dose 240h	None	All	Day 8: post-D1 dose 168h
Weekly Dosing (3 times per week)	Week 3	Day 15: pre-dose, post-dose 6h	D15, D17, D19	All	Day 15: pre-dose
	Week 4	Day 22: pre-dose, post-dose 6h	D22, D24, D26	All	Day 22: pre-dose
	Week 5	Day 29: pre-dose (trough), post-dose 6h	D29, D31, D33	All	Day 29: pre-dose
	Week 6	None	D36, D38, D40	None	None
	Week 7	Day 43: pre-dose (trough), post-dose 6h	D43, D45, D47	All	Day 43: pre-dose
	Week 8	Day 50: pre-dose (trough), post-dose 6h	D50, D52, D54	All	Day 50: pre-dose
	Week 9	Day 57: pre-dose	D57, D59,	All	Day 61: pre-dose

	Week	Sampling Times	Dosing[2]	PK, PPi and PLP	ADA
		Day 61: pre-dose (trough), post-dose 6h, 12h, 24h, 32h, 36h, 48h, 56h, 60h	D61		
No Dosing	Week 10	Day 64: post-D61 dose 72h Day 65: post-D61 dose 96h Day 66: post-D61 dose 120h Day 67: post-D61 dose 144h Day 68: post-D61 dose 168h	None	All	Day 68: post-D61 dose 168h
	Week 11	Day 71: post-D61 dose 240h Day 75: post-D61 dose 336h	None	All	Day 75: post-D61 dose 336h
	Week 12	Day 80: post-D61 dose 456h	None	All	Day 80: post-D61 dose 456h
	Week 13	Day 87: post-D61 dose 624h	None	All	Day 87: post-D61 dose 624h

Abbreviations: ADA = anti-drug antibodies; PK = pharmacokinetics; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate; SOM = Study Operations Manual; D = day; h = hour, NA=not applicable.
NOTE: Trough is defined as pre-dose concentration (ie, concentration data-point collected just prior to the next dose).
NOTE: Baseline definitions:
• For PD assessments, there are 5 pre-treatment baseline samples (-168, -156, -24, -12, and pre-dose Day 1) to be collected. The average value will be used for Baseline.
• For PK and immunogenicity assessments, Baseline is Day 1 pre-dose sample.
1 Timepoints for pre-dose PK sample collection have a window of -15 minutes and for post-dose PK sample collection have a window of ±15 minutes. For additional instructions, please refer to the SOM.
2 The Week 1, Day 1 single dose must be administered on a Monday, and the multiple dosing in Week 3 through Week 9 must be administered on Mondays, Wednesdays, and Fridays.

9.2. Sample Size, Power, and Randomization

A sample size of 9 patients per cohort (27 patients total) will provide sufficient power (>80%) to detect a difference of 2.3 μM between the 9.0 mg/kg/week cohort compared to the 1.5 mg/kg/week cohort, and between the 6.0 mg/kg/week cohort compared to the 1.5 mg/kg/week cohort in the change from Baseline to pre-3PrdP dose in Week 9 PPi (assuming a SD of 1.5 μM). This is based upon the use of a 2-sided, 2-sample t-test analyzed using a fixed sequence testing procedure with the comparison of the 9.0 mg/kg/week cohort compared to the 1.5 mg/kg/week cohort being performed first, and the hypothesis testing for the second comparison 6.0 mg/kg/week cohort compared to the 1.5 mg/kg/week cohort only being performed if the null hypothesis is rejected for the previous comparison at a significance level of 0.05.

A sample size of 6 patients per cohort (18 patients total) will provide sufficient power (>90%) to detect a difference of 3.2 μM (SD 1.5 μM) using the same assumptions and fixed sequence testing procedure. A difference of 3.2 μM (SD 1.5 μM) was observed in adult pediatric-onset treated patients versus controls in Study ENB-009-10 for the change from baseline to Week 6 in PPi. Between 6 and 9 patients per cohort (18 and 27 patients total) will give sufficient power to detect a mean difference of 2.3 to 3.2 μM (SD 1.5 μM).

27 patients who meet eligibility criteria for study participation will be randomized to 1 of the following 3 cohorts:
1. 0.5 mg/kg initial single dose; multiple dosing starting after 2 weeks at 0.5 mg/kg 3 times per week
2. 2.0 mg/kg initial single dose; multiple dosing starting after 2 weeks at 2.0 mg/kg 3 times per week

3. 3.0 mg/kg initial single dose; multiple dosing starting after 2 weeks at 3.0 mg/kg 3 times per week

Randomization will be stratified by gender to ensure a similar distribution of gender in each cohort. An IXRS central randomization scheme will be employed.

9.3. Technical Specifications for Derived Variables

The following derived data will be calculated prior to analysis.

Age
Age will be presented as the number of years between date of birth and the reference date. The following ages may be computed, with reference dates indicated:

Age and reference date

AGE	REFERENCE DATE
• Age at Diagnosis	• Date of Diagnosis
• Age at First Known Sign/Symptom	• Date of Onset of First Known Sign/Symptom
• Age at Enrollment	• Date of Signing ICF
• Age at First Injection	• Date of First Injection

In cases where only the month and year are provided for a date, the day for the date will be imputed as 15. Missing month will be imputed as June. In cases where the day is observed but the month is missing, the date will be imputed as June 15. In instances when the imputed reference date is earlier than the birth date, the birth date will be used as the reference date.

Time Since Reference date

TIME	REFERENCE DATE
• Time Since First Known Sign/Symptom	• Date of Onset of First Known Sign/Symptom
• Time Since Diagnosis	• Date of Diagnosis

Time Since First Known Sign/Symptom and Time Since Diagnosis are based on first dose date, will each be dichotomized into the following categories: < 10 years and ≥ 10 years.

Definition of Treatment Baseline:
• For PPi and PLP assessments, there are 5 pre-treatment baseline samples (-168, -156, -24, -12h, and pre-dose Day 1) to be collected. The average value will be used for Baseline.
• For antibody, and laboratory assessments, Treatment Baseline is the Day 1 pre-dose sample.
• For Safety assessments (including weight), the last assessment prior to first dose will be used for Treatment Baseline.

Adverse Events
Additional details for the analysis of Adverse Events described in Section 7.3.2:

Treatment-emergent AEs (TEAEs) are events with start dates and start times on or after the date and time of the first study drug dose. If the start date of an AE is partially or completely missing

and the end (stop) date and time of the AE does not indicate that it occurred prior to first dose, then the determination of treatment-emergent status will be based on the following:

If the start year is after the year of the first study drug dose, then the AE is treatment-emergent; else,

If the start year is the same as the year of the first study drug dose and the start month is missing, then the AE is treatment emergent; else if the start month is present and is the same or after the month of the first study drug dose, then the AE is treatment-emergent; else, if the start date is completely missing, then the AE is treatment-emergent.

Pre-Treatment Adverse Events (PTAEs) are the AEs occur after the time of informed consent up through the Run-In Period and prior to the 1st dose.

Patient percentages are based on the total number of treated patients in the particular treatment cohort.

Related AEs are defined as possible, probable or definitely related, or events with missing relationship status. Unrelated AEs are defined as unlikely or not related.

Medications and Therapies:

Concomitant medications/therapies are any events with administration dates and times on or after the date and time of the first study drug dose. If the start date of a medication or therapy is partially or completely missing and the end (stop) date and time of the medication/therapy does not indicate that it occurred prior to first dose, then the determination of concomitant status will be based on the following:

If the start year is after the year of the first study drug dose, then the medication/therapy is concomitant; else,

If the start year is the same as the year of the first study drug dose and the start month is missing, then the medication/therapy is concomitant; else if the start month is present and is the same or after the month of the first study drug dose, then the medication/therapy is concomitant; else, if the start date is completely missing, then the medication/therapy is concomitant.

All other medications/therapies are considered prior medications/therapies and could occur from the 30 days prior to informed consent up through the Run-In Period and prior to the 1st dose.

Normal Reference ranges for PPi and PLP:

PPi (μM):
Male/Female
13-18 years 0.75-4.78
>18 years 1-5.82

PLP (ng/mL):
Male/Female
6-18 years 5.74-61.15
>18 years 2.81-26.70

For observations that fall under the lower limit of quantification (LLOQ), the following values will be used:

Table 4: Values Used for Observations under the Lower Limit of Quantification (LLOQ)

Parameter	**Value Used**
Plasma PPi	0.75µM
Plasma PLP	2.5ng/mL
Serum PTH	.5 x LLOQ