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 Protocol:	16 November 2015

PROTOCOL 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

Sponsor: Alexion Pharma GmbH (Alexion)
Giesshübelstrasse 30
CH – 8045 Zürich, Switzerland
Telephone: PPD
Fax: PPD

Sponsor Contact: PPD
PPD
Alexion Pharmaceuticals Inc.
55 Cambridge Parkway, Suite 800
Cambridge, MA 02142 USA
Telephone: PPD
Telephone (mobile): PPD
E-mail: PPD

Medical Monitor: PPD
Alexion Pharmaceuticals Inc.
352 Knotter Drive
Cheshire, CT 06410 USA
Telephone: PPD
Telephone (mobile): PPD
E-mail: PPD

IND No.: 100,619
EudraCT No.: 2015-003131-35

Date: 16 November 2015

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Phase 2a Randomized, Multi-center, Open-Label, Pharmacokinetic, and Dose Response Study of Asfotase Alfa in Adult Patients with Pediatric-Onset Hypophosphatasia

PROTOCOL NUMBER: AA-HPP-208

PPD

Alexion Pharmaceuticals, Inc.
352 Knotter Drive
Cheshire, CT 06410
USA

Telephone PPD

Facsimile:

17-Nov-2015

Date

INVESTIGATOR'S AGREEMENT

PROTOCOL TITLE: A Phase 2a Randomized, Multi-center, Open-Label, Pharmacokinetic, and Dose Response Study of Asfotase Alfa in Adult Patients with Pediatric-Onset Hypophosphatasia

PROTOCOL NUMBER: AA-HPP-208

I have received and read the current Investigator's Brochure for asfotase alfa. I have read the AA-HPP-208 Clinical Study Protocol and agree to conduct the study in accordance with this protocol, all applicable government regulations, the principles of the International Council on Harmonisation (ICH) E6 Guidelines for Good Clinical Practice (GCP), and the principles of the World Medical Association Declaration of Helsinki. I also agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Address and Telephone Number
Clinical Project Lead	PPD	Alexion Pharmaceuticals Inc. 55 Cambridge Parkway, Suite 800 Cambridge, MA 02142 USA Telephone: PPD Telephone (mobile): PPD E-mail: PPD
Medical Monitor (24-Hour emergency contact)	PPD	Alexion Pharmaceuticals Inc. 352 Knotter Drive Cheshire, CT 06410 USA Telephone: PPD Telephone (mobile): PPD E-mail: PPD
Drug Safety Physician	PPD	Alexion Pharmaceuticals Inc. 55 Cambridge Parkway, Suite 800 Cambridge, MA 02142 USA Telephone: PPD Telephone (mobile): PPD Facsimile: PPD E-mail: PPD
24-Hour Serious Adverse Event Contact		Email: PPD *Facsimile: PPD

* Facsimile number is provided as a back-up/contingency for the site to report the serious adverse event in case the site is unable to send the report via email.

1. SYNOPSIS

Name of Sponsor/Company: Alexion Pharma GmbH (Alexion)	
Name of Investigational Product: asfotase alfa	
Name of Active Ingredient: Human recombinant tissue-nonspecific alkaline phosphatase fusion protein	
Title of Study: A Phase 2a Randomized, Multi-center, Open-Label, Pharmacokinetic, and Dose Response Study of Asfotase Alfa in Adult Patients with Pediatric-Onset Hypophosphatasia	
Study center(s): To be determined	
Studied Period: 13.5 Weeks Estimated date first patient enrolled: Q1 2016 Estimated date first patient completed: Q2 2016	**Phase of Development: 2a**
Objectives: Primary Objective The primary objective of this study is 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 mg/kg/week) in adult patients with pediatric-onset hypophosphatasia (HPP). Secondary Objective The secondary objective of this study is to evaluate the safety and tolerability of asfotase alfa in adult patients with pediatric-onset HPP.	
Methodology: Study AA-HPP-208 is a multicenter, randomized, open-label, PK, and PD dose response Phase 2a study of asfotase alfa in adult patients with pediatric-onset HPP. Approximately 27 patients who meet eligibility criteria for study participation will be randomized to 1 of the following 3 cohorts, with the aim of having at least 6 patients in each cohort completing the study: 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. Patients will receive a single dose on Week 1, Day 1. On Week 3, Day 1 patients will start multiple dosing with the 3 times per week regimen (2 weeks after receiving the single dose). Safety, PK, and PD assessments will be performed at Baseline and at scheduled visits throughout the study. 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 due to a safety concern. Adverse events (AEs) and concomitant medications/therapies will be monitored continuously throughout the study.	
Number of Patients Planned: Screened: 36 Randomized: 27 Completed: 18 (minimum)	
Diagnosis and Main Criteria for Inclusion: Men and non-pregnant women aged ≥18 years with a confirmed diagnosis of pediatric-onset HPP. In addition, eligible patients will have either documented tissue-nonspecific alkaline phosphatase (TNSALP) gene mutation(s) or Screening assessments demonstrating both alkaline phosphatase (ALP) below normal and pyridoxal-5′-phosphate (PLP) above normal, and have not received asfotase alfa in the 3 years prior to this study	
Investigational Product, Dosage and Mode of Administration: Asfotase alfa: • 0.5 mg/kg (initial single dose); multiple dosing starting after 2 weeks at 0.5 mg/kg 3 times per week, given as subcutaneous (SC) injections	

- 2.0 mg/kg (initial single dose); multiple dosing starting after 2 weeks at 2.0 mg/kg 3 times per week, given as SC injections
- 3.0 mg/kg (initial single dose); multiple dosing starting after 2 weeks at 3.0 mg/kg 3 times per week, given as SC injections

Individual dose injections are limited by volume to 1.0 mL; those patients whose calculated dose, based on the 3 times per week regimen, exceeds 1.0 mL will require multiple injections per dose.

Duration of Treatment Period:

13.5 weeks: 1 week of run-in, 9 weeks of dosing, and 3.5 weeks of wash-out for immunogenicity assessment

Reference therapy, dosage and mode of administration:

Not applicable

Criteria for evaluation:

Pharmacokinetics:

Blood samples will be drawn at selected timepoints to assess asfotase alfa activity in serum.

Efficacy/Pharmacodynamics:

Blood samples will be drawn at selected timepoints to assess the effect of treatment on PPi and PLP levels. The change from Baseline in PPi and PLP will be evaluated.

Safety:

Adverse events (includes serious adverse events [SAEs], injection site reactions [ISRs], injection associated reactions [IARs]), laboratory tests (clinical chemistry, hematology, and urinalysis), vital signs, physical examination, renal ultrasound, ophthalmology assessment, and urine pregnancy tests (for women of childbearing potential only). Anti-drug antibodies (ADA) and neutralizing antibodies (NAb) will also be assessed.

Statistical methods:

All data collected in this study will be documented using summary tables, figures, and data listings. For categorical variables, frequencies and percentages will be presented. For continuous variables, descriptive statistics (n, mean, median, standard deviation [SD], minimum, and maximum) will be presented. Descriptive statistics for PK parameters will include the number of observations, mean, SD, coefficient of variation (CV), median, minimum, maximum, geometric mean, and geometric %CV.

Efficacy analyses will be conducted on the Full Analysis Set (FAS), consisting of all treated patients with a pre-dose and at least 1 post-dose PPi level. Safety analyses will be conducted on the Safety Set, consisting of all patients who received at least 1 dose of asfotase alfa. Pharmacokinetic analyses will be conducted on the PK Population, consisting of all treated patients for whom the PK profile can be adequately characterized.

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 cohorts in the change from Baseline to pre-3rd dose in Week 9 PPi, assuming a SD of 1.5 μM and 2-sided, 2-sample t-test analyzed using a fixed sequence testing procedure. A sample size of 6 patients per cohort (18 patients total) will provide sufficient power (>90%) to detect a difference of 3.2 μM using the same assumptions. A mean 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).

Efficacy/Pharmacodynamics:

The primary endpoint, PPi change from Baseline to pre-3rd dose in Week 9, will be analyzed using a Wilcoxon rank-sum test. A fixed sequence testing procedure will be performed 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. The primary endpoint will be met if the null hypothesis is rejected for both comparisons at a significance level of 0.05. Additionally, the shift

in the distributions and 95% confidence interval (CI) for the above comparisons will be reported using the Hodges-Lehmann-Sen estimate and the exact confidence limits reported.

As secondary analyses, descriptive statistics for the absolute levels and the change from Baseline in PPi will be summarized at all study timepoints by cohort. Within cohort, the change from Baseline to all study timepoints will be analyzed using the Wilcoxon signed-rank test.

Finally, a restricted maximum likelihood (REML)-based repeated measures mixed model will be fitted to estimate the change from Baseline in PPi at each pre-dose timepoint and test whether the change differs from zero at each timepoint. The analysis will include the fixed, categorical effect of visit. The estimate of change from Baseline at defined visits will be provided, along with 95% CIs and p-values. The treatment effect will also be explored.

For the secondary endpoint, change from Baseline to pre-3$^{rd.}$ dose in Week 9 in PLP, similar methods as for PPi will be used for analysis.

Individual and mean (±SD) plasma PPi and PLP concentration versus time data will be presented graphically by cohort. Additional analysis of the PD data may be performed if considered useful.

Pharmacokinetics:

Mean serum asfotase alfa concentrations versus nominal time and individual serum asfotase alfa concentrations versus actual time will be graphically presented. The individual serum concentration data for asfotase alfa-treated patients, with actual sampling dates and times, will be used to derive the PK parameters by non-compartmental analyses using WinNonlin® 5.3 (or higher) (Certara, L.P., 1699 S Hanley Road, St Louis MO 63144 USA). The following PK parameters will be calculated using non-compartmental methods: maximum observed serum asfotase alfa concentration (C_{max}), time at which C_{max} is observed (t_{max}), area-under-the-concentration-time curve from time zero to the time of the last observed concentration (AUC_t), area-under-the-concentration-time curve from time zero to infinity (AUC_∞), elimination rate constant (k_e), and half-life ($t_{½}$). Additional PK parameters may be calculated, as appropriate. An attainment of asfotase alfa PK steady state will be evaluated. Dose proportionality and time linearity assessment may be considered. PK-PD relationships may be explored. Additional PK-PD analyses may be considered if deemed useful.

Safety:

The incidence of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) will be summarized by System Organ Class (SOC) and Preferred Term (PT) overall, by severity, and by relationship to study drug. Changes from Baseline in vital signs, laboratory assessments (chemistry, hematology, and urinalysis) will be summarized. Shifts from Baseline in laboratory assessments as well as physical examination, renal ultrasound, and ophthalmology findings will be summarized for all study visits. Immunogenicity data will be tabulated and summarized.

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

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Abbreviation	Definition
ADA	anti-drug antibodies
AE	adverse event
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC_t	area-under-the-concentration-time curve from time zero to the time of the last observed concentration
AUC_∞	area-under-the-concentration-time curve from time zero to infinity
B-hCG	human chorionic gonadotropin, beta subunit
BUN	blood urea nitrogen
C_{max}	maximum observed serum concentration
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CV	coefficient of variation
eCRF	electronic case report form
ePRO	electronic patient-reported outcome
EDC	electronic data capture
EOS	end of study
FAS	full analysis set
F/U	follow-up
GCP	Good Clinical Practice
HPP	hypophosphatasia
IAR	injection-associated reaction
IB	Investigator's brochure
ICF	informed consent form
ICH	International Council on Harmonisation
IEC	independent ethics committee
Ig	immunoglobulin
IRB	institutional review board
ISR	injection site reaction
IXRS	interactive voice-/web-response system
K_e	elimination rate constant
mmHg	millimeters of mercury
nAb	neutralizing antibodies
PD	pharmacodynamics
PDA	personal digital assistant
PEA	phosphoethanolamine
PK	pharmacokinetics
PLP	pyridoxal-5′-phosphate

Abbreviation	Definition
PP	per protocol
PPi	inorganic pyrophosphate
PRO	patient-reported outcome
PT	Preferred Term
PTH	parathyroid hormone
REB	Research Ethics Board
REML	restricted maximum likelihood
SAE	serious adverse event
SC	subcutaneous(ly)
SOC	System Organ Class
SOM	study operations manual
SOP	standard operating procedures
SD	standard deviation
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2}$	half-life
T_{max}	time of maximum observed serum concentration
TEAE	treatment-emergent adverse event
TNSALP	tissue-nonspecific alkaline phosphatase
TPO	third party organization

4. INTRODUCTION

Hypophosphatasia (HPP) is a rare metabolic bone disease caused by loss of function mutation(s) in the gene encoding tissue-nonspecific alkaline phosphatase (TNSALP) (Whyte 2013), resulting in defective bone mineralization and impaired phosphate and calcium regulation. Hypophosphatasia is characterized by interdependent clinical manifestations emanating from a failure to mineralize bone matrix. Extracellular accumulation of the TNSALP substrate, inorganic pyrophosphate (PPi), functionally impairs skeletal mineralization. Elevations in extracellular PPi inhibit bone mineralization by blocking hydroxyapatite crystal formation, causing a pronounced accumulation of unmineralized bone matrix. Failure to mineralize bone matrix in adults results in diverse complications that may include proximal muscle weakness, pain, and fractures, including multiple, recurrent, non-healing and non-traumatic fractures (Whyte 2013; Berkseth 2013). Hypophosphatasia is also characterized by lower than normal circulating levels of alkaline phosphatase (ALP) and higher than normal levels of the TNSALP substrates phosphoethanolamine (PEA) and pyridoxal 5'-phosphate (PLP) (Whyte 1985).

In adults, TNSALP activity plays a critical role in bone remodeling, ensuring adequate mineralization of the osteoid matrix, preventing fractures, pseudofractures, and other sequelae of osteomalacia that may lead to profound muscle weakness, decreased physical function, and increasing disability. The adult skeleton undergoes continuous remodeling throughout the entire lifespan to:

- Provide the calcium and phosphorus necessary for metabolism
- Provide the means by which the skeleton can adapt to biomechanical requirements
- Remove old, micro-damaged bone and replace it with new, stronger bone

As bone remodels throughout life, osteoid is deposited at the sites of remodeling. The disease process in HPP inhibits bone mineralization at these sites of remodeling and results in “packets” of soft osteoid. As remodeling continues, the number of these sites on the skeleton with unmineralized areas of osteoid accumulate (ie, osteomalacia), bone strength is reduced, and the integrity of the skeleton is compromised. This compromised skeleton in HPP patients is prone to fractures. The number of fractures and pseudofractures experienced by HPP patients accumulates and increases with age.

The clinical manifestations of HPP range in severity; however, in general there is increased disease severity with an earlier age at onset of disease symptoms. Classifications of HPP described in the literature include pediatric-onset HPP (comprised of perinatal-, infantile-, and juvenile-onset HPP), where first symptoms of HPP present at <18 years of age, and adult-onset HPP, where symptoms appear at ≥18 years of age (Whyte 2013).

Severe functional deficits are commonly present in adults diagnosed with HPP (of any onset) including ambulation difficulties, weakness, shortened stature, and an inability to carry out activities of daily living. In HPP patients surviving to adulthood, long-term clinical sequelae include arthritis, pain, inability to remove previously placed internal fixation devices due to the risk of recurrent fracture, and the requirement for ambulatory assistive devices (eg, wheelchairs,

wheeled walkers, and canes). The most common clinical features reported in adults diagnosed with HPP are musculoskeletal pain and fractures (Berkseth 2013).

Asfotase alfa is a human recombinant tissue-nonspecific alkaline phosphatase-Fc-deca-aspartate fusion protein. It is a soluble glycoprotein of 726 amino acids made from the catalytic domain of human TNSALP (Swiss-Prot, P05186), the human immunoglobulin (Ig) G1 Fc domain (Swiss-Prot, P01857) (to facilitate purification), and a deca-aspartate peptide domain (Kasugai 2000; Nishioka 2006).

Asfotase alfa is an enzyme replacement therapy designed to address the underlying cause of HPP, a deficiency of TNSALP activity, by replacing the defective enzyme and preventing or reversing the mineralization defects of the skeleton, thereby preventing serious patient morbidity. Results from clinical studies to date demonstrate that asfotase alfa treatment was safe and well tolerated and elicits clinically meaningful improvements across a broad array of serious morbidities in HPP, including severely abnormal biochemistry, defective bone mineralization, abnormal bone structure, diminished physical function, and disability.

More information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) of asfotase alfa may be found in the Investigator's Brochure (IB).

5. OBJECTIVES

5.1. Primary Objective

The primary objective of this study is 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 mg/kg/week) in adult patients with pediatric-onset HPP.

5.2. Secondary Objectives

The secondary objective of this study is to evaluate the safety and tolerability of asfotase alfa in adult patients with pediatric-onset HPP.

6. INVESTIGATIONAL PLAN

Study AA-HPP-208 is a multicenter, randomized, open-label, PK, and PD dose response Phase 2a study of asfotase alfa in adult patients with pediatric-onset HPP.

6.1. Overall Study Design

Approximately 27 patients who have provided written informed consent, and have met all the inclusion criteria and none of the exclusion criteria, will be enrolled in the study, with the aim of having at least 6 patients in each cohort completing the study.

Patients will be seen for a Screening visit and will be evaluated after the patient (or legal guardian) provides written informed consent. The Screening visit will include assessments to confirm the diagnosis of HPP, including medical history to confirm onset of HPP symptoms prior to age 18. Patients who fail any of the eligibility criteria may be re-screened only after discussion with the Medical Monitor.

After completion of the Screening visit, patients who meet inclusion/exclusion criteria for Study AA-HPP-208 will be randomized in a 1:1:1 ratio to 1 of the following 3 cohorts:

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

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

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

Randomization will be stratified by gender to ensure a similar distribution of gender in each cohort.

Patients will receive a single dose on Week 1, Day 1; on Week 3, Day 1 (Study Day 15) patients will start multiple dosing with the 3 times per week regimen as described above. All doses will be administered as subcutaneous (SC) injections throughout the study.

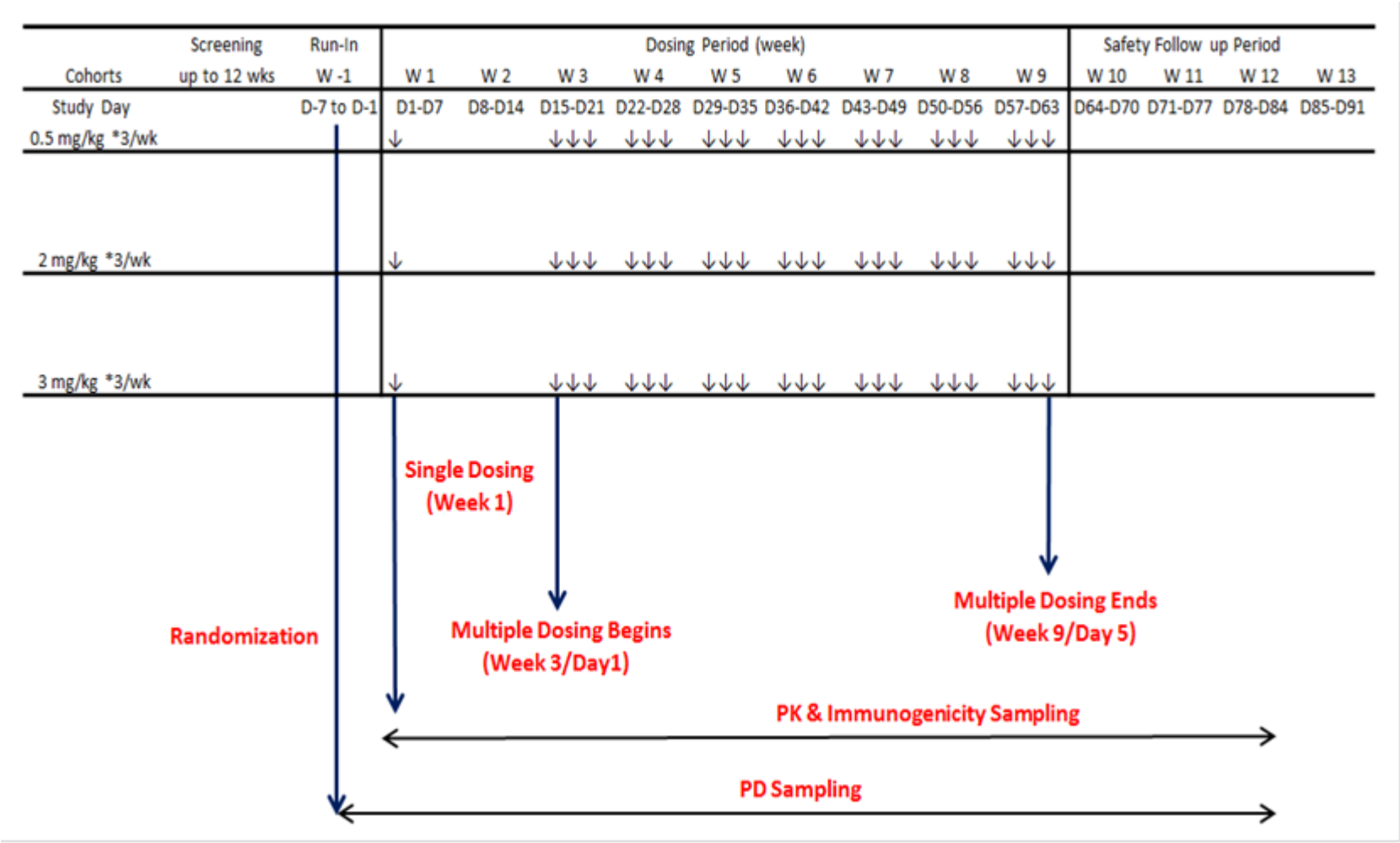
NOTE:

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 Week 3 through Week 9, as indicated in Table 2 and Table 3.

The dose regimens planned for this study (ie, 1.5 mg/kg/week, 6.0 mg/kg/week, and 9.0 mg/kg/week) bracket the recommended dose regimen in pediatric HPP patients of 6.0 mg/kg/week, and span a 6-fold dose range from the lowest to the highest dose, allowing the best opportunity to construct dose-response relationships in adult patients. Weight-based (ie, mg/kg) dosing for asfotase alfa allows normalization of dosing across patient populations with different body weights (inter-patient) and within the same patient over time (intra-patient).

Refer to Figure 1 for an illustration of the study design.

Figure 1: Protocol AA-HPP-208 Study Design



↓ = Dose administration (SC injection)

6.2. Assessments

The study will be conducted as outlined in Table 2 and Table 3. Study visits (in-clinic or inpatient) will occur during Screening (Week -12 to Week -2), the Run-in Period (Week -1), Week 1, Week 9, and Week 13. To facilitate scheduling of required study assessments at each visit, visits may be shortened or prolonged, as necessary, as long as the specified order of assessments and/or availability of results pertaining to laboratory testing and vital signs are adhered to. Following are important points to remember where study assessments are concerned:

- The fasting blood and urine samples are to be collected first (8-hour fast with water ad libitum), before any additional study-related procedures are performed. Once all fasting laboratory samples are collected, patients are allowed to eat and/or drink at will.
- Prior to study drug administration, results of same-day pregnancy testing for females of child-bearing potential must be negative.

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. Laboratory samples collected for routine blood and urine testing will be processed and/or analyzed by a central laboratory. Urine samples for pregnancy testing in female patients of childbearing potential will be analyzed at local laboratories at investigational sites, and blood tests for injection-associated reactions (IARs) occurring following home administration may be analyzed centrally, at the site or other local laboratories as available.

As blood samples taken for study-related testing are sometimes not entirely depleted for the analyses, patients or their legal representative(s) will be given the option to consent to the use of the remaining portion of these samples for additional research. These samples will be used only for Alexion's scientific research related to exploratory biology and biomarker assessments for asfotase alfa treatment and/or HPP. Each sample may continue to be labeled with the patient's study identifiers (ie, patient ID). The patient or legal representative(s) may request that his or her samples, if still identifiable, be destroyed at any time; however, any data already collected from that sample will still be used for this research. The biological samples will remain the property of Alexion, and may be shared with other researchers as long as confidentiality is maintained.

Further details of the study procedures and assessments are provided in the Study Operations Manual (SOM).

Table 2: Schedule of Events

Assessment	Screening[5]	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/ EOS[4] (W13)	
Informed Consent[5]	X										
Inclusion/ Exclusion Criteria	X										
Demographics	X										
Medical history[7]	X										
HPP gene mutation analysis[8]	X										
Concomitant medications[9]	X	X	X	X	X	X	X	X	X	X	
Concomitant therapies[9]	X	X	X	X	X	X	X	X	X	X	
Physical examinations[10]	X	X	X					X		X	
Vital signs[11]	X	X	X	X	X	X	X	X	X	X	
Body Weight[10]	X	X	X		X	X	X	X	X	X	
Randomization		X									
Required In-Clinic[12]	X	X	X					X		X	
Study drug administration-Single Dose[13,14]			X								
Study drug administration-3x Weekly Dosing[13,14]					X	X	X	X			
Chemistry, hematology, urinalysis[15]	X		X					X		X	

Assessment	Screening[5]	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/ EOS[4] (W13)	
Lab Tests (vitamin D, serum PTH, urine Ca:creatinine)[15]	X		X					X		X	
Pregnancy Test[13]	X	X	X					X		X	
Renal ultrasound		X								X	
Ophthalmology exam[16]		X								X	
Pharmaco-dynamics (PPi and PLP)[17,18]	X	X	X	X	X		X	X	X	X	
Anti-drug antibodies[17,18]			X	X	X		X	X	X	X	
Pharmacokinetics[16,17]			X	X	X		X	X	X	X	
Tryptase for IAR[19]			X								
Adverse events[20]		X – *Continuous Monitoring*									
Electronic diary[21]			*X - ContinuousMonitoring*								

Abbreviations: EOS = end-of-study; F/U = follow-up; AE = adverse event;; IAR = injection-associated reaction; PD = pharmacodynamic; PK = pharmacokinetics; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate; PTH = parathyroid hormone; SOM = Study Operations manual; W = Week; D = day.

23TU**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.

NOTE: Baseline definitions:

- For PD assessments, there are 5 pre-treatment baseline samples (-168, -156, -24, -12, and 0h) to be collected. The average value will be used for Baseline.
- For PK, antibody, and laboratory assessments, Baseline is Day 1 pre-dose sample.
- For Safety assessments, the last assessment prior to first dose will be used for Baseline

1. The Safety Follow-Up telephone call will occur 90 days 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 in Week 3 through Week 9 must be administered on Mondays, Wednesdays, and Fridays.

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. The patient's Week 13 visit will be considered their End of Study visit. If a patient who withdraws or discontinues from the study prior to Week 13, a separate End of Study visit will be scheduled.
5. If the time period between the initial Screening and the re-Screening is >12 weeks, all Screening procedures must be repeated.
6. 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.
7. Medical history includes general medical history and HPP-specific medical history, including detail of pediatric-onset. Details of loss of adult teeth will be included as part of medical history.
8. PHPP gene mutation analysis will be performed during Screening or the Rn-In Period if results are not available in the medical records.
9. 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.
10. 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).
11. 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.
12. Required PIn-Clinic or inpatient study visits will occur during Screening, the Run-In Period (Week -1), Week 1, Week 9, and Week 13. At the discretion of the Investigator, Week 2 through Week 8 and Week 10 through Week 12 visits may be conducted in the clinic or at home by a home healthcare provider.
13. Urine and serum pregnancy testing is required for women of childbearing potential only. Serum pregnancy testing is required at Screening and EOS visits; pregnancy testing at Run-In Period, Day 1 visit, and Week 9 visit can be either urine or serum testing at the discretion of the Investigator. Results of pregnancy testing must be negative prior to study drug administration (when applicable).
14. 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.
15. 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.
16. 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.
17. 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).
18. Refer to Table 3 for timing of PK/PD and asfotase alfa anti-drug antibody assessments.
19. Serum sample for tryptase will be collected pre-dose on Day 1. Samples will be drawn only if the patient has no active allergies at the time, and will only be analyzed if the patient experiences a subsequent IAR. 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 as described in Section 9.4.2.1.1 and detailed in the SOM.
20. For systemic hypersensitivity reactions, additional blood and urine samples must be collected to assess the reaction. Refer to Section 9.5 for details of sample collection.
21. Refer to SOM for additional information.

Table 3: Schedule of Events – Pharmacokinetic, Pharmacodynamic, and Immunogenicity Assessments

	Week	Sampling Times	Dosing[2]	PK, PPi and PLP	ADA
Run-In Period	Week -1	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 Day 61: pre-dose (trough), post-dose 6h, 12h Day 62:post-D61 dose 24h, 32h, 36h, Day 63:post-D61 dose 48h, 56h, 60h	D57, D59, D61	All	Day 61: pre-dose
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

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 0h) 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.

7. STUDY POPULATION

7.1. Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for participation in this study:

1. Patients or their legal representative(s) must provide written informed consent prior to undergoing any study-related procedures
2. Patient has pediatric-onset HPP, defined as onset of first sign(s)/symptom(s) of HPP prior to 18 years of age
3. Patients must have a documented diagnosis of HPP as indicated by a documented history of HPP-related skeletal abnormalities and 1 or more of the following:
   - Documented TNSALP gene mutation(s) from a certified laboratory
   - Serum ALP level below the age-adjusted normal range AND plasma PLP above the upper limit of normal at Screening.

   NOTE: Historical results from a certified laboratory for PLP may be used to determine patient eligibility. The criterion for plasma PLP is not applicable if the patient is receiving pyridoxine treatment.
4. Patients must have a plasma PPi level of ≥3.9 μM at Screening
5. Patients must be ≥18 years of age at Screening
6. Sexually active male and female patients of childbearing potential must agree to use a highly effective method of birth control during the study and for 3 months following the last dose of study drug. Male patients must also not donate sperm during the Screening and treatment periods and for at least 3 months after the last dose of asfotase alfa. Highly effective contraceptive methods are as follows:
   a. Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
      - Oral
      - Intravaginal
      - Transdermal
   b. Progestogen-only hormonal contraception associated with inhibition of ovulation:
      - Oral
      - Injectable
      - Implantable
   c. Intrauterine device
   d. Intrauterine hormone-releasing system
   e. Bilateral tubal occlusion

f. Vasectomy with documented medical assessment of surgical success
g. Condom and spermicide

7. Female patients of childbearing potential must have a negative pregnancy test at the time of enrollment
8. Female patients not of child-bearing potential due to surgical sterilization (at least 6 weeks after surgical bilateral oophorectomy with or without hysterectomy or at least 6 weeks after tubal ligation) confirmed by medical history, or menopause
   a. Menopausal women include women with either
      - Spontaneous amenorrhea for at least 12 months, not induced by a medical condition such as anorexia nervosa and not taking medications during the amenorrhea that induced the amenorrhea (for example, oral contraceptives, hormones, gonadotropin releasing hormone, antiestrogens, selective estrogen receptor modulators, or chemotherapy), or
      - Spontaneous amenorrhea for 6 to 12 months and a follicle-stimulating hormone level >40 mIU/mL or estradiol level ≤110 pmol/L
9. Patients must be willing to comply with study procedures and the visit schedule

7.2. Exclusion Criteria

Patients will be excluded from participation in this study if they meet any of the following exclusion criteria:

1. Investigational site personnel directly affiliated with this study and/or their immediate families. Immediate family is defined as a spouse, parent, child, or sibling, whether biological or legally adopted.
2. Employees of Alexion Pharmaceuticals
3. Currently enrolled in a clinical study involving another study drug or nonapproved use of a drug or device, or concurrently enrolled in any other type of medical research judged not to be scientifically or medically compatible with this study (excluding the HPP registry, in which concurrent enrollment is allowed).
4. Participated, within the last 30 days, in a clinical study involving a study drug (other than the study drug used in this study). If the previous study drug has a long half-life, 3 months or 5 half-lives (whichever is longer) should have passed.
5. Have completed or withdrawn from this study or any other study investigating asfotase alfa in the previous 3 years. This exclusion criterion does not apply to patients who are re-screened prior to randomization or patients enrolled in the HPP registry.
6. Women who are pregnant, planning to become pregnant, or breastfeeding
7. Serum 25-OH Vitamin D below 20 ng/mL at Screening (results from local laboratory may be used if within 4 weeks of Screening)
8. Screening serum creatinine or parathyroid hormone (PTH) levels 1.5 times the upper limit of normal

9. Medical condition, serious concurrent illness and/or injury, recent orthopedic surgery, or other extenuating circumstance that, in the opinion of the Investigator, may significantly interfere with study compliance or study endpoints, including all prescribed evaluations and follow-up activities
10. Prior treatment with bisphosphonates within 2 years of study entry for any length of time or for more than 2 consecutive years at any prior timepoint
11. Treatment with PTH, strontium, or sclerostin inhibitors within 6 months prior to the first dose of study drug
12. Unwilling or unable to comply with the use of a data collection device on which study patients will directly record data

7.3. Discontinuations

7.3.1. Discontinuation of Patients

The criteria for enrollment must be followed explicitly. If the investigational site identifies a patient who did not meet enrollment criteria and who was inadvertently enrolled, the Sponsor must be notified. If the Sponsor identifies a patient who did not meet enrollment criteria and who was inadvertently enrolled, the investigational site will be notified. A discussion must occur between the Sponsor's Medical Monitor and the Investigator to determine whether the patient may continue in the study, with or without study drug. Ineligible patients who are inadvertently enrolled may be maintained in the study and on study drug when the Sponsor's Medical Monitor agrees with the Investigator that it is medically appropriate for that patient. The patient may not continue in the study with or without study drug if the Sponsor's Medical Monitor does not agree with the Investigator's determination that it is medically appropriate for the patient to continue. The Investigator must obtain documented approval from the Sponsor's Medical Monitor to allow the inadvertently enrolled patient to continue in the study with or without study drug.

In addition, patients will be discontinued from the study drug and/or from the study in the following circumstances:

- Enrollment in any other clinical study involving a study drug or enrollment in any other type of medical research judged not to be scientifically or medically compatible with this study
- Investigator Decision
  - The Investigator decides that the patient should be discontinued from the study
  - If the patient, for any reason, requires treatment with another therapeutic agent that has been demonstrated to be effective for treatment of HPP, discontinuation from the study will occur prior to introduction of the new agent
- Patient Decision
  - The patient requests to be withdrawn from the study

- Sponsor Decision
  - Alexion or designee stops the study or stops the patient's participation in the study for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and Good Clinical Practice (GCP)
- Adverse Event
  - If the Investigator decides that the patient should be withdrawn because of a serious AE (SAE) or a clinically significant laboratory value, the study drug is to be discontinued and appropriate measures are to be taken. Alexion or designee is to be alerted immediately. Refer to Section 9.4.1.11.

Patients who discontinue the study drug and/or study early will have end-of-study procedures performed as shown in the Schedule of Events (Table 2).

All AEs ongoing at the time of withdrawal require 30 day follow-up. The Investigator will be asked to follow all SAEs that were ongoing at the time of withdrawal until resolution or until the patient is lost to follow-up.

7.3.2. Discontinuation of Study Sites

Study site participation may be discontinued if Alexion or designee, the Investigator, or the institutional review board, independent ethics committee, or research ethics board (IRB/IEC/REB) of the study site judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

7.3.3. Discontinuation of Study

The study will be discontinued if Alexion or designee judges it necessary for medical, safety, regulatory, or other reasons consistent with applicable laws, regulations, and GCP.

8. TREATMENT

8.1. Treatments Administered

This study involves an evaluation of asfotase alfa administered by SC injection at doses of thrice-weekly 0.5 mg/kg (1.5 mg/kg/week), 2.0 mg/kg (6.0 mg/kg/week), and 3.0 mg/kg (9.0 mg/kg/week). Asfotase alfa should be administered only as a SC injection and should not be administered intravenously or intramuscularly. The maximum volume of medicinal product per injection should not exceed 1.0 mL. If more than 1.0 mL is required, multiple injections may be administered at the same time. Table 4 presents the treatment regimens.

Table 4: Treatment Regimens

Cohort	Regimen and Dose
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
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
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

The total amount of asfotase alfa administered may be adjusted at each study visit to account for changes in body weight.

The Investigator or designee is responsible for the following:

- Explaining the correct use of the study drug to the patient or legal representative
- Verifying that instructions are followed properly
- Maintaining accurate records of study drug dispensing and collection
- Returning all unused study drug to the Sponsor or designee at the end of the study

8.2. Clinical Study Materials and Supplies

Asfotase alfa is a fusion protein made from the soluble catalytic domain of human TNSALP, the human IgG1 Fc domain for purification, and a deca-aspartate peptide used as a bone targeting domain. It is derived from Chinese Hamster Ovary cells and is supplied as a clear, colorless, sterile solution for injection in single use glass vials.

Table 5 summarizes the concentrations and extractable amounts of study drug that will be used for the study.

Table 5: Summary of Vial Concentrations and Extractable Volumes

Concentration	Extractable Volume (mL)	Extractable Product (mg)
100 mg/mL	0.8	80

At a minimum, study drug will be labeled with:

- The protocol number
- Lot number/expiry date

- Sponsor name and address
- Instructions for use and storage

Study drug will be labeled according to the country's regulatory requirements.

Study drug must be refrigerated between 2°C and 8°C in a secure area with limited access immediately upon receipt and inventory at the investigational site. To ensure the integrity of the study drug, storage temperature at the investigational site must be monitored on a daily basis and appropriately documented throughout the study conduct. Study drug administered for home use by patients should be stored refrigerated at 2 C to 8°C.

The following guidelines must be adhered to where study drug handling is concerned:

- The Investigator must only supply study drug to patients enrolled in the clinical study
- The Investigator must ensure study drug is stored under controlled storage conditions, accessible only to those authorized by the Investigator to dispense the study drug
- The Investigator must ensure that study drug accountability is maintained on an ongoing basis during the study
- At the conclusion or termination of the study, after final accountability for study drug inventory has been performed, the Investigator must ensure unused study drug supplies at the investigational site are returned to Alexion or designee in accordance with Sponsor instructions or destroyed if the site has appropriate facilities and written procedures to dispose of study drug

Additional information on study drug accountability, handling, and disposal requirements can be found in the Pharmacy Manual.

Note: In some cases, sites may be permitted to destroy the study drug if, during the investigational site selection, the study monitor has verified and documented that the site has appropriate facilities and written procedures to dispose of study drug. The material must first be counted, reconciled, and approved for destruction by the Sponsor or designee.

8.3. Method of Assignment to Treatment

Patients who meet all criteria for enrollment will be randomized to a cohort during Run-In Period (Week -1). Assignment to cohorts will be determined by a computer-generated random sequence using an interactive voice- or web- response system (IXRS). Enough vials for the calculated doses between visits will be dispensed to study patients at each visit.

Approximately 27 adult patients who meet eligibility criteria for study participation will be randomized to in a 1:1:1 ratio to 1 of the following 3 cohorts:

- Cohort 1: 0.5 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 0.5 mg/kg 3 times per week
- Cohort 2: 2.0 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 2.0 mg/kg 3 times per week
- Cohort 3: 3.0 mg/kg initial single dose; followed by multiple dosing starting after 2 weeks at 3.0 mg/kg 3 times per week dosing

Randomization will be stratified by gender to ensure a similar distribution of gender in each cohort.

8.4. Rationale for Selection of Doses in the Study

Based on relevant non-clinical data, as well as current human experience with asfotase alfa in patients with hypophosphatasia to date, a dose of 6 mg/kg/week administered subcutaneously is the recommended dose in pediatric patients; this dose regimen has been approved for treatment of pediatric patients with HPP in the US, EU, and other countries.

The dose regimens planned for this study (ie, 1.5 mg/kg/week, 6.0 mg/kg/week, and 9.0 mg/kg/week) bracket the recommended dose regimen in pediatric HPP patients and span a 6-fold dose range from the lowest to the highest dose, allowing the best opportunity to construct dose-response relationships in adult patients with pediatric-onset HPP.

Weight based (ie, mg/kg) dosing for asfotase alfa allows normalization of dosing across patient populations with different body weights (inter-patient) and within the same patient over time (intra-patient).

8.5. Selection and Timing of Doses

Patients will be randomly assigned by IXRS to 1 of the 3 cohorts (Table 4). Weekly doses of asfotase alfa will be administered 3 days per week as detailed in Section 8.1.

The number of vials used to prepare injections will be determined based on the patient's weight at that visit. The first dose of asfotase alfa will be prepared and administered at the investigational site on the Week 1, Day 1 study visit. Asfotase alfa will also be prepared and administered at the investigational site on dosing days that occur during all required study visits. The Investigator, patient, or designee may prepare and administer injections at study visits. For injections between study visits, asfotase alfa may be prepared and administered by the patient or designee at home under the supervision of home healthcare.

If a patient experiences an acute or severe IAR at home (eg, with signs/symptoms of hypersensitivity, irrespective of the time from administration of study drug to onset), the patient or legal representative(s) should call the site as soon as possible to discuss with the Investigator the need for medical evaluation in the medical office/hospital. Collection of blood samples for analyses outlined in Section 9.5 may be required upon recommendation of the Investigator and availability of these tests at the local laboratory.

Throughout the study, injection sites should be rotated among 8 different body areas (both thighs and buttocks, both sides of the abdomen, and both upper arms) and carefully monitored for sign(s) of potential reaction(s). Note that any injection sites with unresolved injection site reactions (ISRs) should not be used for injections again until the reaction subsides.

Full instructions for data collection using the electronic diary are included in the SOM.

8.6. Blinding and Unblinding

This is an open-label study.

8.7. Concomitant Medication/Therapy

Any HPP-related medication use by the patient within 30 days prior to study entry through the final study visit must be recorded. Concomitant medications, including pretreatment with antihistamines or steroids, for patients who experience IARs will be monitored continuously throughout the study.

Local anesthesia should not be used when administering injections of asfotase alfa because the interactions of a local anesthetic and asfotase alfa are unknown.

8.8. Treatment Compliance

The Investigator or designee must ensure that all study participants are adequately informed on the specific study drug regimens required for compliance with the study protocol.

Study drug compliance will be determined by the following:

- Study drug administration data will be entered into the electronic patient diary by the patient and reviewed by the Investigator at each study visit
- Missed and improperly administered study drug doses must be clearly documented in the patient's electronic diaries and study records, and documented as protocol deviations
- The patients will be instructed to return any used study drug at the next study visit for the purpose of performing drug accountability

For drug accountability purposes, empty vials of asfotase alfa will be discarded in a sharps container and returned when the container is full, or at the last study visit.

Treatment compliance is defined as taking 100% of the required doses of study drug; missed doses will be captured as a protocol deviation. Study sites are encouraged to make telephone calls to patients at regular intervals to ensure patient compliance with their assigned injectable study drug.

In addition to the assessment of a patient's compliance with the study drug administration, other aspects of compliance with the study treatments will be assessed at each visit based on the patient's adherence to the visit schedule, completion of study diaries, and any other parameters the Investigator considers necessary.

9. PHARMACOKINETIC AND PHARMACODYNAMIC ASSESSMENTS, SAFETY EVALUATIONS, SAMPLE COLLECTION AND TESTING, AND APPROPRIATENESS OF MEASUREMENTS

Study procedures and their timing (including tolerance limits for timing) are summarized in the Schedule of Assessments (Table 2). Details of the analyses to be performed for each assessment are summarized in Section 11.

NOTE:

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 Week 3 through Week 9, as indicated in Table 2 and Table 3.

9.1. Pharmacokinetic Assessments

9.1.1. Asfotase Alfa Activity

Assessment of PK will be performed on all patients. Blood samples for the measurement of serum asfotase alfa activity will be collected at the visits and/or timepoints specified in Table 2 and Table 3 (timepoints for pre-dose PK sample collection have a window of -15 minutes, and post-dose PK sample collection have a window of ±15 minutes). Serum asfotase alfa activity will be measured at a central laboratory. Refer to the SOM and Laboratory Manual for complete PK collection procedures as well as preparation and shipping procedures.

9.2. Efficacy/Pharmacodynamic Assessments

9.2.1. Plasma Inorganic Pyrophosphate and Plasma Pyridoxal-5' Phosphate

Blood samples for measurement of PPi and PLP in plasma will be collected at the study visits and times indicated in Table 2 and Table 3 to assess as potential substrates of asfotase alfa activity. These collections will only be made using levamisole tubes as outlined in the Laboratory Manual.

9.3. Safety Assessments

9.3.1. Physical Examinations

A physical examination will be performed at the times specified in Table 2 using a standardized form with a checklist of items. Each examination will include the following assessments: general appearance; skin; head, ear, eye, nose, and throat (including tooth count); neck; lymph nodes; chest; heart; abdominal cavity; limbs; central nervous system; and musculoskeletal.

If clinically significant changes from Screening/Baseline are noted, the changes will be documented as AEs on the AE tab of the electronic Case Report Form (eCRF). Clinical significance is defined as any variation in physical findings that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the patient until

the parameter returns to Baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.3.2. Vital Signs

The following vital signs will be recorded at the times specified in Table 2: height (cm), weight (kg), 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]). Vital signs will be taken immediately prior to each study drug administration.

If clinically significant vital sign changes as compared to Screening/Baseline are noted, the changes will be documented as AEs on the AE tab of the eCRF. Clinical significance is defined as any variation in vital signs that has medical relevance and may result in an alteration in medical care. The Investigator will continue to monitor the patient until the parameter returns to Baseline or until the Investigator determines that follow-up is no longer medically necessary.

9.3.3. Serum Chemistry, Hematology, and Urinalysis

Serum chemistry, hematology, and urinalysis will be performed at the times specified in Table 2. Specific laboratory assessments are provided in Appendix 14.1.

Laboratory assessments for safety will be tested at a central laboratory facility. Laboratory reports will be made available to the Investigators in a timely manner for clinical management of patients.

The Investigators must assess all abnormal laboratory values as either clinically significant or not clinically significant. It is anticipated that some laboratory values may be outside of the normal value range due to the underlying disease. As in routine practice, the Investigators should use their medical judgment when assessing clinical significance. Clinical significance is defined as any variation in laboratory measurements which has medical relevance and which results in a change in medical care. If clinically significant laboratory changes from Baseline are noted, the changes will be documented as AEs on the AE tab of the eCRF. The Investigator will also assess the relationship to study treatment for all clinically significant out of range values (Section 9.4.1.8). The Investigator will continue to monitor the patient with additional laboratory assessments until (1) values have reached normal range and/or Baseline, or (2) in the judgment of the Investigator, out of range values are not related to the administration of study drug or other protocol-specific procedures.

9.3.4. Ophthalmology Assessments

A full ophthalmology examination will be performed at the times specified in Table 2. 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. Sites will be provided ophthalmologic worksheets required to complete the full exam. Ophthalmology exams may be performed by a qualified ophthalmologist or optometrist (ie, Doctor of Optometry, OD) as long as the optometrist works under the supervision of an ophthalmologist (MD).

Clinically significant changes from Baseline in ophthalmology examinations must be considered an AE and recorded in the eCRF.

Additional information on ophthalmology examinations can be found in the SOM.

9.3.5. Renal Ultrasound

A renal ultrasound will be performed as outlined in Table 2 to assess for the presence of nephrocalcinosis. Clinically significant changes from Baseline in renal ultrasounds must be considered an AE and recorded in the eCRF for the study.

9.4. Safety Evaluations

Investigators are responsible for monitoring the safety of patients and for alerting Alexion or designee to any event that seems unusual, even if this event may be considered an unanticipated benefit to the patient.

The Investigator is responsible for detecting, assessing, documenting, and reporting of all AEs.

Adverse Events reported by the patient, by the parent or legal guardian, identified in response to an open-ended question from study personnel, or revealed by observation, physical examination, or other study procedures must be collected and recorded as described in Section 9.4.1.4.

9.4.1. Adverse Event

An AE is any untoward medical occurrence in a patient or clinical study subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (for example, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to this medicinal product.

Exacerbation of a chronic or intermittent pre-existing condition including an increase in frequency and/or intensity of the condition, and abnormal laboratory findings that are considered to be of clinical significance are all considered to be AEs.

The definition of an AE also covers medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital), and anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen are not AEs.

High ALP levels detected through clinical laboratory testing will not be considered an AE after initiation of the study drug as these reflect circulating levels of asfotase alfa.

9.4.1.1. Medical Procedures

Elective procedures that were pre-planned prior to the time that written informed consent was obtained are not considered to be AEs. Any complication or worsening of a pre-existing condition leading to the procedure is considered an AE. In addition, any AE that occurs as an outcome of the planned procedure is considered an AE.

Diagnostic and therapeutic procedures (invasive and non-invasive) such as surgery or angiography should not be reported as an AE or SAE. However, the medical condition or the diagnosis that was responsible for the procedure should be recorded. The procedure should be recorded in the narrative as treatment for the AE or SAE (eg, laparoscopic cholecystectomy is the procedure or treatment for an SAE of necrotic gall bladder).

9.4.1.2. Abnormal Test Findings

Abnormal test findings may be considered AEs or SAEs; however, Investigators are strongly encouraged to report the diagnosis, sign, or symptom rather than the abnormal result. The criteria for an abnormal test finding being classified as an AE or SAE are any of the following:

- Test result is associated with a sign or symptom
- Test result requires additional diagnostic testing
- Test result requires a medical or surgical intervention
- Test result leads to a change in study dosing outside of the protocol defined dosing or discontinuation from the study
- Test result requires significant additional treatment (ie, addition of new medication, significant increase in dose of current medication)

9.4.1.3. Lack of Efficacy

Lack of drug effect is not an AE in clinical studies, because the purpose of the clinical study is to establish drug effect.

9.4.1.4. Recording Adverse Events

All observed or reported AEs regardless of treatment or causal relationship must be recorded and reported as described in Section 9.4.1.11.

For all AEs, the Investigator must obtain adequate information for the following:

1. Determine the outcome of the AE
2. Determine if the event meets criteria for an SAE
3. Assess the severity of the AE
4. Determine the causality of the AE

For all AEs, regardless of casual relationship, the Investigator must follow-up on the outcome of the event until the event or sequelae either resolve or stabilize.

Adverse events spontaneously reported by the patient, and/or parent or legal guardian and/or identified in response to an open-ended question from study personnel, or revealed by observation will be recorded during the study phase by the Investigator. Adverse event information will be collected from the signing of informed consent until completion of the study.

Adverse Events must be documented in clear, unambiguous medical terms. Study personnel are advised not to use abbreviations or acronyms.

For each AE record only the diagnosis on the AE page of eCRF, do not report the characteristic signs and symptoms of the diagnosis as additional AEs.

If a diagnosis is not available, record each sign and symptom as an AE, when a diagnosis becomes available, study personnel are to update the AE page of the eCRF with relevant diagnosis only.

Change in severity of an AE should be documented based on specific guidelines in the eCRF Completion Guidelines.

9.4.1.5. Expectedness Assessment of Adverse Events

The expectedness of an AE is determined by the Sponsor in the reference safety information contained in the current version of the Investigator Brochure (IB).

9.4.1.6. Serious Adverse Events

An AE that fulfills any 1 of the criteria listed below must be recorded as an SAE.

An SAE or reaction is described as any untoward medical occurrence that at any dose:

1. Is a congenital anomaly/birth defect
2. Results in persistent or significant disability/incapacity
3. Results in death
4. Results in or prolongs hospitalization

   NOTE: Requires inpatient hospitalization or prolongation of existing hospitalization. Adverse events that are associated with hospitalization or prolongation of hospitalization are considered SAEs. All admissions to a health care facility meet this criteria, even if less than 24 hours. Criteria for seriousness are also met if transfer within the hospital is done to receive more intense medical or surgical care (eg, medical floor to the ICU).

   Hospitalization does not include the following:

   - Rehabilitation facility
   - Hospice facility
   - Nursing facility
   - Emergency Room
   - Same day surgery
   - Hospitalization or prolongation of hospitalization not associated with an adverse event is not an SAE; examples include admission for a pre-existing condition not associated with either a new AE or with worsening of a pre-existing AE, protocol-specified admission, pre-planned admission
5. Is life-threatening

   NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

6. Other medically important SAE

NOTE: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

9.4.1.6.1. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the IB and that the investigator identifies as related to study drug or procedure. United States 21 CFR 312.32 and European Union Clinical Trial Directive 2001/20/EC and the associated detailed guidances or national regulatory requirements in participating countries require the reporting of SUSARs. The Sponsor has procedures that will be followed for the recording and expedited reporting of SUSARs that are consistent with global regulations and the associated detailed guidances.

9.4.1.7. Severity Assessments

Investigators will be instructed to rate the severity of AEs using the following criteria:

Mild Events require minimal or no treatment and do not interfere with the patient's daily activities.

Moderate Events result in a low level of inconvenience or concerns with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe Events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Change in severity of an AE should be documented based on specific guidelines in the eCRF Completion Guidelines.

Severity and seriousness must be differentiated: severity describes the intensity of an AE, while the term seriousness refers to an AE that has met the criteria for an SAE.

9.4.1.8. Causality Assessment

An Investigator causality assessment (Unrelated, Unlikely, Possible, Probable, or Definite) must be provided for all AEs (both serious and non-serious). This assessment must be recorded in the eCRF and any additional SAE forms as appropriate.

Not Related	Suggests that there is no association between the Investigational Product and the reported event.
Unlikely Related	Suggests that the clinical picture is highly consistent with a cause other than the Investigational Product but attribution cannot be made with absolute certainty and a relationship between the Investigational Product, and AE cannot be excluded with complete confidence.
Possibly Related	Suggests that treatment with the Investigational Product may have caused or contributed to the AE (ie, the event follows a reasonable temporal sequence from the time of drug administration and/or follows a known response pattern to the Investigational Product, but could also have been produced by other factors).
Probably Related	Suggests that a reasonable temporal sequence of the event with the Investigational Product administration exists and there is likely association of the event with the Investigational Product. This will be based upon the known pharmacological action of the Investigational Product, known or previously reported adverse reactions to the Investigational Product or class of drugs, or judgment based on the Investigator's clinical experience.
Definitely Related	Temporal relationship to the Investigational Product, other conditions (concurrent illness, concurrent medication reaction, or progression/expression of disease state) do not appear to explain event, corresponds with the known pharmaceutical profile, improvement on discontinuation, re-appearance on re-challenge.

9.4.1.9. Adverse Event Outcome

If a patient experiences an SAE with an outcome of death:

- The SAE resulting in death should have an outcome documented as death/fatal with an end date as the date of death
- If the patient had additional AEs/SAEs that were ongoing at the time of death, these events should be documented as ongoing with no end date
- Only 1 event should have an outcome of death/fatal unless an autopsy report or Investigator states otherwise

9.4.1.10. Exposure During Pregnancy and/or Lactation

Pregnancy data will be collected during this study for all patients.

For all Alexion products, both in development or post-approval, exposure during pregnancy must be recorded and followed. Exposure during pregnancy, also called exposure in utero, can be the result of either maternal exposure or transmission of drug product via semen following paternal exposure.

If a patient within this study or a patient's partner becomes or is found to be pregnant while treated or exposed to study drug, the Investigator must submit a pregnancy form to Alexion via the same method as SAE reporting. Alexion Global Pharmacovigilance will supply the Investigator with a copy of a "Pregnancy Reporting and Outcome Form/Breast Feeding" form and must be notified via the same method as SAE reporting.

Exposure of an infant to an Alexion product during breastfeeding would need to be reported via the "Pregnancy Reporting and Outcome Form/Breast Feeding" form, and any AEs an infant may experience following breastfeeding needs to be reported to Alexion or Alexion Global Pharmacovigilance designee.

The patient should be followed until the outcome of the pregnancy is known (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality), even if the patient discontinued study drug or discontinues from the study. When the outcome of the pregnancy becomes known the form should be completed and returned to Alexion or Alexion Global Pharmacovigilance designee. If additional follow-up is required, the Investigator will be requested to provide the information.

Pregnancy in itself is not regarded as an AE unless there is a suspicion that study drug may have interfered with the effectiveness of a contraceptive medication. However, complications of pregnancy and abnormal outcomes of pregnancy are AEs and many may meet criteria for an SAE. Complications of pregnancy and abnormal outcomes of pregnancy such as ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly would meet criteria of an SAE and thus, should be reported as an SAE. Elective abortions without complications should not be reported as AEs.

9.4.1.11. Reporting of Adverse Event(s) and Serious Adverse Event(s) to the Sponsor

All non-serious AEs must be recorded in the electronic data capture (EDC) system upon awareness.

All SAEs must be reported to Alexion Pharmacovigilance within 24 hours of first awareness of the event by the Investigator or designee. The reporting timelines must be followed for all initial SAE cases and follow-up reports to the initial case.

The Investigator must record the SAE data in the patient's eCRF and verify the accuracy of the information recorded pages with the corresponding source documents. The SAE information will be sent via the Safety Gateway, a tool within the electronic data capture system, to Alexion Global Pharmacovigilance.

Please note - The fax number and email are provided below as a back-up/contingency for the site to report the SAE in case the site is unable to send the report via Safety Gateway.

Email: PPD

***Fax:** PPD

If applicable or requested, additional information such as relevant medical records and/or diagnostic information should be reported to Alexion Global Pharmacovigilance via the email or fax address as noted above..

Any new follow-up information regarding the SAE should be entered in the patient's eCRF and sent electronically via the Safety Gateway to Alexion Global Pharmacovigilance within the same timeframe as the initial report stated above.

For all SAEs reported via email/fax, the Investigator must provide the following:

- Patient's Registry ID number
- Causality of the SAE(s) to asfotase alfa
- Outcome of the SAE(s) (ie, resolved, resolving, resolved with sequelae, not resolved, or death)
- Relevant medical records and laboratory/diagnostic information (eg, a copy of death certificate and/or autopsy results, if applicable, for SAE reports involving a death)
- Appropriate and requested follow-up information in the time frame detailed above.

Alexion is responsible for notifying the relevant regulatory authorities of certain events. Depending on local regulations, the Sponsor, the Sponsors designee, or the Investigator will be responsible for notifying the IRB/EC/REB of all SAEs that occur at a site per local IRB/EC/REB-established guidelines for submission. Investigators will also be notified of all unexpected, serious, drug-related events that have been expedited to regulatory authorities during the HPP Registry. These additional SAEs will also be reported to sites IRB/EC/REB per local regulations.

9.4.1.12. Blinded studies

This is an open-label study.

9.4.1.13. Sponsor Reporting Requirements

The Sponsor or a legal representative is responsible for notifying the relevant regulatory authorities of SAE's meeting the reporting criteria.

9.4.1.14. Investigator Reporting Requirements

Investigators must fulfill all required local regulatory obligations required for the study conduct.

9.4.2. Other Safety Measures

9.4.2.1. Injection-Related Adverse Events

Injection-related AEs of special interest collected and reported in the asfotase alfa clinical development program are injection-associated reactions (IARs) and injection site reactions (ISRs).

9.4.2.1.1. Injection-Associated Reactions

Injection-associated reactions are defined as systemic signs or symptoms (eg, generalized urticaria or itching, hypotension, or respiratory distress) that occur within 3 hours after study drug administration that are assessed by the Investigator as possibly, probably, or definitely related to study drug.

Adverse events that are characterized as IARs may reflect a systemic hypersensitivity reaction (eg, combination of 2 or more of the following types of signs or symptoms: generalized urticaria or itching, hypotension, difficulty breathing, swelling of the eyelids or lips, or generalized edema). These AEs are to be captured as the individual AE(s) that occurred and are not to be grouped and recorded with the term "IAR" or "hypersensitivity."

For systemic hypersensitivity reactions, the investigational site will collect laboratory samples for analysis of the following:

- Tryptase (serum)
  - Within 1 hour from time of reaction onset, if possible (no longer than 2 hours after onset)
  - At 24 hours (or later) from time of reaction onset
- C5b-9 (EDTA plasma with protease inhibitors [P100 tubes])
  - Within 24 hours from time of reaction onset
- Hematology, blood chemistry, and urinalysis
  - Within 3 hours from time of reaction onset
  - At 24 hours from time of reaction onset

Patients will undergo a one-time pre-dose collection of serum for tryptase analysis on Day 1 prior to administration of study drug, in order to provide a Baseline tryptase value in case of a future IAR. The sample will be drawn only if the patient has no active allergies at the time, and will only be analyzed (along with the above assessments) if the patient experiences a subsequent acute or severe IAR. If the patient has an ongoing active allergic process on Day 1, the tryptase sample should be collected as soon as possible after the resolution of the allergic event.

Treatment guidelines can be found in the SOM.

If a patient experiences a systemic hypersensitivity reaction at home, the patient or legal representative(s) should call the site as soon as possible to discuss with the Investigator the need for medical evaluation in a medical facility. Collection of blood samples for the analyses outlined above may be required upon recommendation of the Investigator and availability of these tests at the local laboratory. If the patient lives near the investigational site, the medical evaluation may be done and blood for the tests may be drawn there.

For patients who experience moderate or severe IARs, continuation of study drug injections in the study will be determined by the Investigator in consultation with the Sponsor's Medical Monitor. In the case of a favorable benefit-risk assessment, where the decision is made to continue study drug injections, an individualized pretreatment strategy with antihistamines or

steroids will be developed as necessary at the discretion of the Investigator. Should such pretreatment medications be used, they should be recorded as concomitant medications.

All IARs, whether serious or non-serious, must be recorded on the eCRF. If any sign(s) and/or symptom(s) of an IAR are considered serious, the Alexion Global Pharmacovigilance group or designee must be notified in accordance with the procedures outlined in Section 9.4.1.11.

9.4.2.1.2. Injection Site Reactions

Injection site reactions (ISRs) are defined as AEs localized to the site of study drug administration that occur at any time during study participation that are assessed by the Investigator as possibly, probably, or definitely related to study drug.

Injection site reactions may occur at any timepoint after study drug administration. There is no additional laboratory testing required for ISRs.

The Investigator is responsible for monitoring for ISRs in patients throughout the duration of the study. As part of the study-required physical examinations, Investigators should evaluate injection sites for signs of reaction(s). All ISRs, whether non-serious or serious, must be recorded on the eCRF. If any ISRs are considered serious, the Alexion Global Pharmacovigilance group or designee must be notified in accordance with the procedures outlined in Section 9.4.1.11.

9.4.2.2. Anti-Drug Antibodies

Patients will be monitored for development of antibody production against asfotase alfa (asfotase alfa anti-drug antibodies [ADA]) throughout this study (Table 2). Serum will be collected and analyzed for the presence of ADA, and if positive further analyzed for titer, neutralizing antibodies, and characterization of the response.

9.4.2.3. Pregnancy and Breastfeeding

The effects of asfotase alfa on conception, pregnancy, and lactation in humans are not known.

All female patients of childbearing potential and sexually mature males will be required to use a medically accepted method of contraception throughout the study and for 3 months following the last dose of study drug. Agreement to this requirement will be captured at the Screening visit as part of the consent procedure and patients will be followed until 3 months after the last dose of study drug.

If a female patient becomes pregnant during the treatment period of the study, asfotase alfa treatment must be immediately discontinued. See Section 9.4.1.10 for details on handling of pregnancies during the study.

9.5. Sample Collection and Testing

Table 2 lists the schedule for sample collections in this study.

Appendix 14.1 lists the laboratory tests that will be performed for this study.

Appendix 14.2 provides a summary of the maximum number and volume of invasive samples for all sampling during the study.

9.5.1. Samples for Study Qualification and Health Monitoring

Blood and urine samples will be collected to determine whether patients meet inclusion/exclusion criteria and to monitor patient safety.

Investigators must document their review of each laboratory safety report.

Tests are run and confirmed promptly whenever scientifically appropriate. However, when scientific circumstances warrant it is acceptable to retain samples to batch the tests run or to retain the samples until the end of the study to confirm that the results are valid. Certain samples may be retained for a longer period, if necessary, to comply with applicable laws, regulations, or laboratory certification standards.

As blood samples taken for study-related testing are sometimes not entirely depleted for the analyses, patients or their legal representative(s) will be given the option to consent to use of the remaining portion of these samples for additional research. These samples will be used only for Alexion's scientific research related to exploratory biology and biomarker assessments for asfotase alfa treatment and/or HPP. Each sample may continue to be labeled with the patient's study identifiers (ie, patient ID). The patient or legal representative(s) may request that his or her samples, if still identifiable, be destroyed at any time; however, any data already collected from that sample will still be used for this research. The biological samples will remain the property of Alexion, and may be shared with other researchers as long as confidentiality is maintained.

9.5.2. Samples for Immunogenicity

Blood samples for immunogenicity testing will be collected to determine antibody production against asfotase alfa at the visits and times specified in the Schedule of Events (Table 2 and Table 3), venous blood samples of approximately 2.5 mL each will be collected to determine the serum ADA level. Immunogenicity will be assessed by a validated assay designed to detect ADA in the presence of asfotase alfa. Antibodies may be further characterized and/or evaluated for their ability to neutralize the activity of asfotase alfa.

Samples may be stored for a maximum of 15 years following last patient visit for the study at a facility selected by the Sponsor to enable further analysis of immune responses to asfotase alfa. The duration allows the Sponsor to respond to regulatory requests related to asfotase alfa.

9.5.3. Samples for Drug Concentration Measurements

At the visits and times specified in the Schedule of Events (Table 2 and Table 3), venous blood samples of approximately 2.5 mL each will be collected to determine the serum activity of asfotase alfa. Instructions for the collection and handling of blood samples will be provided in the Laboratory Manual. The actual date and time (24-hour clock time) of each sampling will be recorded.

Bioanalytical samples collected to measure study drug concentration will be retained for a maximum of 1 year following last patient visit for the study.

9.6. Appropriateness of Measurements

All efficacy and safety assessments included in this study are generally regarded as reliable and accurate with respect to the efficacy and safety assessments in individuals with HPP.

10. DATA QUALITY ASSURANCE

To ensure accurate, complete, and reliable data, the Sponsor or its representatives will do the following:

- Provide instructional material to the study sites, as appropriate
- Provide start-up training to instruct the Investigators and study coordinators. This training will give instruction on the protocol, the completion of the eCRFs, and study procedures.
- Make periodic visits to the study site
- Be available for consultation and stay in contact with the study site personnel
- Review and evaluate eCRF data and use standard computer edits to detect errors in data collection
- Conduct a quality review of the database

In addition, the Sponsor or its representatives will periodically check a sample of the patient data recorded against source documents at the study site. The study may be audited by the Sponsor or its representatives, and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of participants in the study, and to ensure accurate, complete, and reliable data, the Investigator will keep records of laboratory tests, clinical notes, and patient medical records in the patient files as original source documents for the study. If requested, the Investigator will provide the sponsor, applicable regulatory agencies, and applicable IRB/IEC/REB(s) with direct access to original source documents.

10.1. Data Capture System

An EDC system will be used in this study. The site must maintain a separate source for the data entered into the EDC system.

Electronic patient-reported outcome (ePRO) measures or other data reported directly by the patient (for example, daily dosing schedule) are entered into an ePRO instrument (for example, personal digital assistant [PDA], or by means of IXRS) at the time that the information is obtained. The ePRO device will be used to collect date including, but not necessarily limited to, dosing data; refer to the Study Operations Manual for further information. In these instances where there is no prior written or electronic source data at the site, the ePRO instrument record will serve as the source.

Any data for which the ePRO instrument record will serve to collect source data will be identified and documented by each site in that site's study file.

All data from the medical records of qualifying patients will be entered into eCRFs designed by Alexion or designee. Electronic CRFs will be completed by investigational study site personnel or their designee. Electronic CRF completion guidelines describing the procedures for recording

data on the eCRFs will be provided to the investigational sites and training on the guideline and use of the EDC system will occur prior to eCRF completion.

Any data for which paper documentation provided by the patient will serve as the source document will be identified and documented by each site in that site's study file. Paper documentation provided by the patient may include, for example, a paper diary to collect patient-reported outcome (PRO) measures.

11. STATISTICS

Alexion will be responsible for data collection and editing, reviewing and validating all the information in the eCRFs, statistical analysis, and generation of the clinical study report.

Prior to locking the database, all data editing will be completed and decisions regarding the evaluability of all patient data for inclusion in the statistical analysis will be made. The rationale for excluding any data from the statistical analyses will be prospectively defined, and classification of all or part of a patient's data as non-evaluable will be completed and documented before the database is locked and before the statistical analysis is begun. The statistical analysis will not begin until the entire database is locked and signed off, in accordance with the Standard Operating Procedures (SOPs) of the Alexion Biometrics Department.

Alexion or designee will perform the statistical analysis of the data derived from this study. The analysis will be performed using the SAS® statistical software system Version 9.2 or later. Pharmacokinetic parameters will be estimated using WinNonlin®5.3 (or higher) (Certara, L.P., 1699 S Hanley Road, St Louis MO 63144 USA).

11.1. Analysis Populations

11.1.1. Full Analysis Set

The Full Analysis Set (FAS) consists of all patients who are randomized to treatment and received at least 1 dose of study drug and have at least 1 pre-treatment and 1 on-treatment PPi result. All analyses of PD/efficacy will be performed based on the FAS. Patients will be included in the analyses according to the treatment cohort to which they were randomized, irrespective of the treatment they actually received. The efficacy analyses based upon the FAS will be considered the primary analysis of efficacy.

11.1.2. Per Protocol Set

The Per Protocol (PP) population or PP Set consists of all patients in the FAS Population with no major protocol deviations. Major protocol deviations will include:

- Non-compliance with study treatment
- Not receiving all doses of study drug
- 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 done prior to the database lock. All analyses of PD/efficacy will also be performed based on the PP 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. The efficacy analyses based upon the PP Set will be considered secondary analyses of efficacy.

11.1.3. Safety Set

All patients who receive any amount of study drug treatment will comprise the Safety Set. Patients will be considered, for safety analysis, to be in the treatment cohort of the treatment they actually received.

11.1.4. Pharmacokinetic Population

All treated patients for whom the PK profile can be adequately characterized will comprise the PK Population. The PK Population will be used for the analysis of PK data. PK analyses will be based upon the treatment actually received.

11.2. Efficacy/Pharmacodynamic Endpoints

11.2.1. Primary Efficacy/Pharmacodynamic Endpoint

The primary efficacy/PD endpoint will be the change in PPi from Baseline to pre-3rd dose in Week 9. Baseline and pre-3rd dose Week 9 are defined in Section 11.5.4.

11.2.2. Secondary Efficacy/Pharmacodynamic Endpoints

The secondary efficacy/PD endpoint will be the change in PLP from Baseline to pre-3rd dose in Week 9. Baseline and pre-3rd dose Week 9 are defined in Section 11.5.4.

11.3. Pharmacokinetics

The PK data from patients will be tabulated and summary statistics will be reported for each cohort.

The following PK parameters will be calculated using non-compartmental methods:

- Maximum observed serum concentration (C_{max})
- Time of maximum observed serum concentration (t_{max})
- Area under the concentration-time curve from time zero to time of the last observed concentration (AUC_t)
- Area under the concentration-time curve from time zero to infinity (AUC_∞)
- Elimination rate constant (k_e)
- Half-life ($t_{½}$)

Additional PK parameters may be calculated, as appropriate.

11.4. Safety Endpoints

Safety variables include the incidence of AEs (includes SAEs; ISRs; IARs; discontinuations due to AEs; and drug-related serious and severe AEs), as well as clinical and laboratory tests (clinical chemistry, hematology, and urinalysis), vital signs (blood pressure, heart rate, respiratory rate, and temperature), physical examinations, renal ultrasound, ophthalmology assessment, pregnancy testing (for women of childbearing potential), asfotase alfa anti-drug antibodies (ADA), and neutralizing antibodies (nAb).

11.5. Statistical Methods

All data collected in this study will be documented using summary tables, figures, and patient data listings. For categorical variables, frequencies and percentages will be presented for each cohort and for the combined cohorts (where appropriate). For continuous variables, descriptive statistics (n, mean, median, standard deviation [SD], minimum, maximum) will be presented for each cohort and for the combined cohorts (where appropriate). Descriptive statistics for PK parameters will include the number of observations, mean, SD, coefficient of variation (CV), median, minimum, maximum, geometric mean, and geometric %CV. All hypothesis testing will be 2-sided and performed at the 0.05 level of significance.

Pharmacodynamic/efficacy analyses will be conducted on the FAS, consisting of all treated patients with at least 1 pre-treatment and 1 on-treatment PPi value. Additionally, PD/efficacy analyses will be conducted on the PP Set. Pharmacokinetic analyses will be conducted on the PK population, consisting of all treated patients for whom the PK profile can be adequately characterized. Safety analyses will be conducted on the Safety Set, consisting of all patients who received at least 1 dose of asfotase alfa.

For the PD/efficacy analyses, patients will be considered to be in the treatment cohort to which they were randomized. For safety and PK analyses, patients will be considered to be in the treatment cohort of the treatment they actually received.

11.5.1. Handling of Dropouts/Missing Data

For each patient, there are 5 pre-treatment baseline samples (-168, -156, -24, -12, and 0h) to be collected. The average value will be used for analysis. Therefore, at least 1 pre-treatment baseline observation is required for the patient to be included for the assessment of the primary PD/efficacy analysis (PPi). If a patient lacks a pre-treatment value they will be excluded from the analysis. In addition, patients will be required to have at least 1 on-treatment observation. Missing values at the pre-3rd dose Week 9 assessment will be imputed using last observation carried forward (LOCF). This imputation will occur only for the primary endpoint.

Missing or invalid safety data will not be replaced.

11.5.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics including gender, ethnicity, age at study treatment initiation, age at initial symptoms, age at diagnosis, and time since initial symptom onset will be summarized by cohort and overall using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) or frequencies and percentages, as appropriate. All data will be presented in by-patient data listings.

Medical/surgical history will be summarized descriptively by cohort and overall using counts and percentages and will also be presented in by-patient data listings.

Concomitant medication/therapy data and pre-treatment medication data will be presented in listings. Concomitant medications will also be summarized in tabular form by cohort and overall using frequencies and percentages. The WHO Drug dictionary will be used to code the medications.

11.5.3. Patient Accountability

All patients randomized to treatment will be included in the summary of patient disposition and accountability overall and by cohort. A listing of patients indicating randomization to treatment, attendance at each visit, discontinuation from the study, and completion of the study will also be generated.

11.5.4. Efficacy/Pharmacodynamic Analyses

11.5.4.1. Primary Efficacy/Pharmacodynamic Endpoint

The primary efficacy/PD endpoint will be the change from PPi Baseline to pre-3rd dose Week 9. Baseline is defined as the average of all available Run-in Period assessments on or prior to first SC injection with asfotase alfa. Pre-3rd dose Week 9 is defined as the value obtained prior to the third dose during Week 9. To assess differences between cohorts, the Wilcoxon rank-sum test will be used. A fixed sequence testing procedure will be performed 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. The primary endpoint will be met if the null hypothesis is rejected for both comparisons at a significance level of 0.05. Additionally, the shift in the distributions and 95% confidence interval (CI) for the above comparisons will be reported using the Hodges-Lehmann-Sen estimate and the exact confidence limits reported.

As secondary analyses, descriptive statistics for absolute PPi level and the change from Baseline will be summarized at all study timepoints by cohort. Within cohort, the change from Baseline to all study timepoints will be analyzed using the Wilcoxon signed-rank test.

Additionally, a restricted maximum likelihood (REML)-based repeated measures mixed model will be fit to estimate the change from Baseline in PPi at each pre-dose timepoint during the study period and test whether the change differs from zero at each timepoint. The analysis will include the fixed, categorical effect of visit. 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 estimate of change from Baseline at defined visits will be provided, along with 95% CIs and p-values. The treatment effect will also be explored.

Individual and mean concentration versus time curves will be presented. Additional analysis of PPi data may be performed if considered useful.

11.5.4.2. Secondary Efficacy/Pharmacodynamic Endpoints

For the secondary/PD endpoint, PLP change from Baseline to pre-3rd dose Week 9, similar methods as for PPi will be employed.

11.5.5. Statistical Methods: Pharmacokinetics Analysis

Following dosing at Week 1, Day1, mean serum asfotase alfa concentrations versus *nominal* time, and individual serum asfotase alfa concentrations versus *actual* time will be graphically presented for both linear and semi-log scales. The individual serum concentration data for patients in each cohort, with actual sampling dates and times will be used to derive the PK parameters by non-compartmental analyses using WinNonlin®5.3 (or higher) (Certara, L.P., 1699 S Hanley Road, St Louis MO 63144 USA). The following PK parameters will be calculated and summary statistics reported by cohort and by patient: C_{max}, t_{max}, $AUC_{t,}$ AUC_{∞}, k_e, and $t_{½}$. Both the single-dose and multiple-dose PK will be characterized. Descriptive statistics (mean, SD, CV, median, minimum, maximum, geometric mean, and geometric %CV) of the serum concentration and PK parameters will be presented. An attainment of asfotase alfa PK steady state will be evaluated. Dose proportionality and time linearity assessment may be considered. Graphical displays of PK data will be presented as appropriate.

Relationships between PK and PD parameters may be explored if considered appropriate. The PK and PD data from this study may be combined with data from other studies.

11.5.6. Statistical Methods: Safety Variables

Summaries for all safety variables will be computed and displayed by cohort.

11.5.6.1. Safety Variable: Adverse Events

Safety assessments will be based on the incidence of AE reports. Adverse events will be coded using MedDRA and will be summarized by System Organ Class (SOC) and Preferred Term (PT). A detailed listing of patients who experience AEs and SAEs will be presented. Adverse event severity will be categorized as mild, moderate, or severe. The highest severity will be assigned to a patient should more than 1 occurrence of the same AE be reported with a different severity. Relationships of the AE to treatment will be categorized as not related/unrelated, unlikely related, possibly related, probably related, or definitely related. The highest level of association will be reported in patients with differing relationships for the same AE. A listing of AEs for all patients will be provided. A separate listing for patients who withdraw from the study due to AEs will be provided. The incidence of AEs will be tabulated by severity, and by relationship to treatment. The incidence of AEs leading to study discontinuation will also be summarized. Adverse events occurring on the day of infusion will be summarized, and a separate listing will be produced for these AEs. All summary tables and data listings of AEs will be presented by cohort.

11.5.6.2. Safety Variable: Injection-Associated Reactions

Injection-associated reactions will be analyzed in the same manner as AEs.

11.5.6.3. Safety Variable: Injection Site Reactions

Injection site reactions will be analyzed in the same manner as AEs.

11.5.6.4. Safety Variable: Laboratory Tests Including Clinical Hematology, Chemistry, and Urinalysis

Actual values and changes from Baseline in quantitative lab values will be summarized by study cohort and timepoint using descriptive statistics. All laboratory values will be classified as normal, below normal, or above normal based on normal ranges supplied by the laboratory. An analysis of laboratory values will be based on frequencies of abnormal values and frequencies of clinically significant abnormal values and will be presented in tabular form by cohort. Shift tables will be produced. All data will be presented in listings by cohort along with individual patient listings for those patients with clinically significant abnormal laboratory values. Graphical displays will be presented as appropriate.

11.5.6.5. Safety Variable: Asfotase Alfa Anti-drug Antibody

Shift tables will be produced to show changes from Baseline to scheduled visits in the presence or absence of asfotase alfa ADA. For patients with positive antibodies, the titer level will be summarized using descriptive statistics, and the presence or absence of neutralizing antibodies will be summarized using counts and percentages. These analyses will be performed by cohort. Graphical displays will be presented as appropriate.

11.5.6.6. Safety Variables: Vital Signs, Physical Examinations, Renal Ultrasounds, and Ophthalmology Assessments

Actual values and changes from Baseline in vital signs (blood pressure, heart rate, respiratory rate, and temperature) will be summarized by cohort and timepoint using descriptive statistics. Physical examination (including weight), renal ultrasound, and ophthalmology findings will be summarized by cohort and timepoint using counts and percentages. Listings of abnormal results will be provided.

11.5.7. Statistical Methods: Study Drug Administration

Study drug administration data will be presented by cohort in a listing.

11.5.8. Statistical Methods: Power and Sample Size

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-3rd 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).

11.5.9. Statistical Methods: Multiplicity

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.

12. INFORMED CONSENT, ETHICAL REVIEW, AND REGULATORY CONSIDERATIONS

12.1. Informed Consent

The Investigator is responsible for ensuring that the patient understands the potential risks and benefits of participating in the study, including answering any questions the patient may have throughout the study and sharing in a timely manner any new information that may be relevant to the patient's willingness to continue participation in the study.

The Informed Consent Form (ICF) will be used to explain the potential risks and benefits of study participation to the patient in simple terms before the patient is entered into the study, and to document that the patient is satisfied with his or her understanding of the risks and benefits of participating in the study and desires to participate in the study.

The Investigator is responsible for ensuring that informed consent is given by each patient or legal representative. This includes obtaining the appropriate signatures and dates on the ICF prior to the performance of any protocol procedures and prior to the administration of study drug.

12.2. Ethical Review

The Sponsor or its representatives must approve all ICFs before they are used at investigational sites(s). All ICFs must be compliant with the International Council on Harmonisation (ICH) guidelines on GCP.

Documentation of IRB/IEC/REB approval of the protocol and the ICF must be provided to the Sponsor before the study may begin at the investigational site(s). The IRB/IEC/REB(s) will review the protocol as required.

The study site's IRB/IEC/REB(s) should be provided with the following at a minimum:

- The current IB and updates during the course of the study
- ICF
- Relevant curricula vitae

12.3. Regulatory Considerations

This study will be conducted in accordance with:

1. Consensus ethics principles derived from international ethics guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
2. The ICH GCP Guideline [E6]
3. Applicable laws and regulations

The Investigator or designee will promptly submit the protocol to applicable IRB/IEC/REB(s).

Some of the obligations of the Sponsor may be assigned to a third party organization (TPO).

An identification code assigned by the IXRS to each patient will be used in lieu of the patient's name to protect the patient's identity when reporting AEs and/or other study-related data.

12.3.1. Investigator Information

Physicians with experience in metabolic bone disease will participate as investigators in this clinical study.

12.3.2. Protocol Signatures

The Sponsor's responsible medical officer will approve the protocol, confirming that, to the best of his or her knowledge, the protocol accurately describes the planned design and conduct of the study.

After reading the protocol, each Investigator will sign the protocol signature page and send a copy of the signed page to a Sponsor representative.

12.3.3. Final Clinical Study Report Signature

The Principal Investigator will sign the final clinical study report for this study, indicating agreement that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

The Sponsor's responsible medical officer and statistician will approve the final clinical study report for this study, confirming that, to the best of his or her knowledge, the report accurately describes the conduct and results of the study.

12.4. Publication Policy

The full terms regarding publication of the results of this study are outlined in the Clinical Study Agreement, Statement of Agreement, or the Master Clinical Study Agreement. Publication is permitted only after multi-center results are available and all disclosure requirements for clinical study registries have been met. Any data to be submitted for publication, including abstract submissions or presentations, are required to be submitted to Alexion for review at least 30 days prior to submission.

The process for identifying the coordinating Investigator will include evaluation of relevant criteria including, but not limited to: 1) enrollment of the greatest number of patients; 2) clinical experience with HPP; 3) demonstrated understanding of, and adherence to, this protocol; and 4) contribution to study development and/or implementation.

13. LIST OF REFERENCES

1. Berkseth KE, Tebben PJ, Drake MT, Hefferan TE, Jewison DE, Wemers RA. Clinical spectrum of hypophosphatasia diagnosed in adults. Bone 2013;54:21-7.

2. Kasugai S, Fujisawa R, Waki Y, Miyamoto K, Ohya K. Selective drug delivery system to bone: small peptide (Asp)6 conjugation. J.Bone Miner.Res. 2000 May;15(5):936-43.

3. Nishioka T, Tomatsu S, Gutierrez GA, Miyamoto K, Trandafirescu GG, Lopez PLC, et al. Enhancement of drug delivery to bone: Characterization of human tissue-nonspecific alkaline phosphatase tagged with an acidic oligopeptide. Molecular Genetics and Metabolism 2006 Feb 23;88(3):244-55.

4. Whyte MP. Hypophosphatasia. In: Thakker RV, Whyte MP, Eisman J, et al., editors. Genetics of Bone Biology and Skeletal Disease. San Diego: Academic Press; 2013. p. 337-60.

5. Whyte MP, Mahuren JD, Vrabel LA, Coburn SP. Markedly Increased Circulating Pyridoxal-5'-Phosphate Levels in Hypophosphatasia. J Clin Invest 1985;76:752-6.

14. APPENDICES

14.1. Study AA-HPP-208: Clinical Laboratory Tests

Protocol AA-HPP-208: Clinical Laboratory Tests

Hematology[a]:	***Clinical Chemistry[a]:***
Erythrocytes	Sodium
Hemoglobin	Potassium
Hematocrit	Total bilirubin
Red blood cell indices	Direct bilirubin
Mean corpuscular volume reticulocytes	Indirect bilirubin
Ret. mean corpuscular hemoglobin	Alkaline phosphatase
Ret. corpuscular hemoglobin content	Alanine aminotransferase (ALT)
Reticulocyte distribution width	Aspartate aminotransferase (AST)
White blood cell count and differential	Blood urea nitrogen (BUN)
Neutrophils, segmented	Creatinine
Lymphocytes	Urea
Monocytes	Calcium
Eosinophils	Phosphate
Basophils	Glucose, fasting
Platelets	Albumin
Mean platelet volume	Protein
Urinalysis[a]:	Carbon dioxide
pH	Magnesium
Protein	Chloride
Glucose	
Ketones	***Other Laboratory Tests[a]***
Bilirubin	Parathyroid hormone, intact
Erythrocytes	25-OH Vitamin D
Leukocyte esterase	
Nitrite	***Pregnancy Test*** *(female patients only)[b,c]*
Urobilinogen	Choriogonadotropin Beta
Calcium	
Calcium/Creatinine	
Microscopy	

Abbreviations: ret. = reticulocyte
a Assayed by Alexion-designated laboratory
b Results will be confirmed at Screening
c Local or Investigator-designated laboratory

14.2. Study AA-HPP-208: Sampling Summary

This table summarizes the approximate number of samples and volumes for all sampling and tests during the study. Fewer samples may actually be taken, but this will not require a protocol amendment.

Protocol AA-HPP-208: Sampling Summary per Patient

Purpose	Sample Type	Approximate Amount per Sample	Approximate Number Samples	Approximate Total Amount
Screening tests[a]	Blood	2.5 mL	6	15.0 mL
Standard laboratory tests[a]	Blood	2.5 mL	20	50.0 mL
Pharmacokinetic samples	Blood	2.5 mL	46	115.0 mL
Pharmacodynamic samples[b]	Blood	2.0 mL	96	192.0 mL
Immunogenicity samples	Blood	2.5 mL	12	30.0 mL
Total	Blood	12.0 mL	182	402.0 mL

[a] Additional samples may be drawn if needed for safety purposes.
[b] Plasma inorganic pyrophosphate (PPi) and plasma pyridoxal-5' phosphate (PLP)

16 December 2015

RE: Administrative Change Letter: Protocol Clarifications

Protocol 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

To whom it may concern:

This letter is to clarify the following:

- **Dose adjustment:** The process for dose adjustment is inconsistent in Section 8.1 and Section 8.5 of the protocol. This letter is to clarify that total amount of asfotase alfa will be adjusted once a week at study visits (in-clinic or home) to account for body weight change. The number of vials used to prepare injections will be determined based on the patient's weight at that visit. The same number of vials and total amount of asfotase alfa will be administered until a new weight is recorded.
- **Electronic Diary:** Study drug administration data will be entered into an electronic diary by the home health care nurse or site staff. The protocol has inconsistent description of the process in Sections 8.5, 8.8, and 10.1 and Table 2, footnote 21. Full instructions for data collection using an electronic diary will be provided in the Study Operations Manual.
- **Pregnancy Testing:** Pregnancy testing will be performed during in-clinic visits (Screening, Run-in, Week 1, Week 9, and Week 13). In the protocol, the timing of pregnancy testing is described inconsistently in Section 6.2 and Table 2, footnote 13. This clarification will also be included in the Study Operations Manual.
- **Drug Accountability:** Patients will be instructed to return any used study drug at the next study visit for the purpose of performing drug accountability. This process is described inconsistently in Section 8.8 and will be further clarified in the Pharmacy Manual.
- **Reporting of Serious Adverse Event (SAE):** In Section 9.4.1.11, the protocol lists Patient's Registry ID number as one of the required information for SAE reporting. However, there is no Registry ID number in this study. The information expected is the Patient's ID number.
- **Clinical Laboratory Tests:** Red blood cell indices (which include Mean corpuscular volume reticulocytes, Ret. mean corpuscular hemoglobin, Ret. corpuscular hemoglobin content, Reticulocyte distribution width) and mean platelet volume are listed in the Appendix 14.1; however, these labs were included inadvertently and will not be implemented for this study. Erythrocytes assay is listed under Hematology; however, the intended assay is Red Blood Cell Count and will be clarified in the Laboratory Manual. Additionally, Blood urea nitrogen (BUN) and Urea are listed under Clinical Chemistry; however, the

intended test is Urea nitrogen and will be further clarified in the Laboratory Manual.

- **Nomenclature for Pre-dose Sampling:** The pretreatment/Baseline sampling (prior to the first dose of asfotase alfa) will occur at -168h, -156h, -24h, and immediately prior to the first dose (pre-dose). In Table 2 (first bulleted footnote), Table 3 (first bulleted footnote), and Section 11.5.1, this 'pre-dose' sample is designated as '0h' (Hour 0); however, in all places this sampling time is referred to as 'pre-dose.'
- **Schedule of Events:** Further information on the timing and schedule of assessments will be included in the Study Operations Manual.
- **Fasting Criteria**: Footnote 15 in Table 2 clarifies the protocol requirements for fasting. This requirement as stated in Table 2, footnote 15 also applies to Table 3.

Sincerely,

PPD

Cc: eTMF, ISF
PPD Project Manager

Alexion Pharmaceuticals, Inc.

Protocol 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

Date: 01 April 2016

Administrative Change Letter #2

To: PPD

There have been changes associated with the Study AA-HPP-208 protocol, dated 16 November 2015. Effective immediately, the following changes are in effect:

- The estimated date for first patient enrolled in Study AA-HPP-208 has been extended to Q2 2016.
- The estimated date for first patient completed in Study AA-HPP-208 has been extended to Q3 2016.
- Study drug administration data will be entered into an electronic diary by the home health care nurse or site staff. The protocol has inconsistent description of the process in Sections 7.2, 8.5, 8.8, and 10.1 and Table 2, footnote 21. Full instructions for data collection using an electronic diary will be provided in the Study Operations Manual.
- Screening serum creatinine or parathyroid hormone (PTH) levels ≥1.5 times the upper limit of normal is an exclusion criterion.
- 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.
- All pre-dose PK/PD/ADA samples during the Run-in Period and Main Study Period (Treatment Period) must be collected under fasting conditions, except for Day -7, -156 hour and Day -1, -12 hour samples. The post-dose PK/PD/ADA samples will not be collected under fasting conditions.
- Effective March 3rd, 2016, the Drug Safety Physician of this protocol is
  PPD
  Title: PPD
  Email: PPD
  Phone number: PPD

If there are any questions concerning this change, please contact PPD by phone at PPD or by email at PPD This change will be captured in the next protocol amendment.

Sincerely,

PPD

PPD

Alexion Pharmaceuticals, Inc.
100 College Street, New Haven, CT 06510

Cc: eTMF, ISF
PPD Project Manager