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*APPLICATION NUMBER:*

**125513Orig1s000**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**

## ADDENDUM TO CLINICAL PHARMACOLOGY REVIEW

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BLA	<b>125513 (eCTD sequence 0000)</b>
Drug Name	<b>Asfotase Alfa (Strensiq)</b>
Formulation	<b>Sterile aqueous solution for subcutaneous (SC) injection</b>
Formulation Strength	<b>40 mg/mL and 100 mg/mL</b>
Dosing Regimen	<b>1 mg/kg SC 6 times/weeks or 2 mg/kg SC 3 times/week</b>
Route of Administration	<b>Subcutaneous</b>
Description of Submission	<b>Original NME BLA</b>
Received Date	<b>12/23/2014</b>
PDUFA Date	<b>11/23/2015</b>
Clinical Pharmacology Reviewer	<b>Christine Yuen-Yi Hon, Pharm.D</b>
Clinical Pharmacology Team Lead	<b>Yow-Ming Wang, Ph.D.</b>
OCP Division	<b>DCP3</b>
OND Division	<b>DGIEP</b>
Applicant	<b>Alexion</b>
Proposed Indication	<b>Treatment of Patients with Infantile-Onset and Juvenile-Onset Hypophosphatasia</b>

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This addendum provides updated pharmacokinetic (PK) parameter results for asfotase alfa in Tables 9, 10, and 13 of the Clinical Pharmacology Review dated August 9, 2015. In the initial BLA submission, the Applicant presented the PK parameters and the actual dose administered in terms of enzyme activity unit (i.e., U). In order to facilitate asfotase alfa product labeling, the Clinical Pharmacology Review team requested that the Applicant presents the PK results and actual dose administered in mass unit, i.e., mg/kg, ng/mL, (ng/mL)/(mg/kg), etc. (See Clinical Pharmacology Information Request [IR] dated August 7, 2015). The Applicant provided the response to the IR on August 24, 2015, and the updated PK information of asfotase alfa is summarized below.

### Updated PK Summary Tables 9, 10, and 13

Although the PK data in the original BLA submission were expressed in terms of activity units (U/L), the standard curve range of the PK assays was established in terms of protein concentration (ng/mL) and the results were converted to activity units (U/L) and reported as such. The Applicant converted the protein concentration (ng/mL) to activity units (U/L) using the following formula:

Upon FDA request, the Applicant converted asfotase alfa activity units (U/L) back to mass units (ng/mL) by using the following equation:

As the specific activity of asfotase alfa is required for converting the asfotase alfa concentration data in U/L unit to ng/mL unit and vice versa, the Applicant assigned the specific activity value of the reference standard used for each of the PK assay runs for the data conversion. Two lots of reference standards (FIL094H01 and QC-2178) with specific activities of (b) (4) U/mg, respectively, were used in the PK assay, and the standards were prepared according to the mass unit (ng/mL). In order to retrospectively assign one of these two specific activity values, the Applicant relied on the lower limit of quantification (LLOQ) value of each assay PK run. Specifically, the data were converted using a specific activity of (b) (4) U/mg for samples in the PK assay runs with a LLOQ of 120.6 U/L, and a specific activity of (b) (4) U/mg was applied when the LLOQ of the PK assay run was 126.75 U/L.

The revised PK data provided by the Applicant are summarized in Table 9, 10, and 13 below.

In addition to the unit change mentioned above, PK data from study ENB-010-10 were updated from a total of 12 subjects to 14 subjects in Table 9 and from 15 subjects to 14 subjects in Table 10. Further details are described below.

In the original submission, the Applicant submitted PK data derived from samples up to 32 hours post-dose with the exception of two subjects who had data up to 24-hour post-dose. In the Response to Clinical Pharmacology IR dated August 24, 2015, the Applicant provided additional PK data obtained up to 48 hours post-dose; therefore, all subjects had 48-hour PK profiles. Consequently, the updated Table 9 contain two more subjects compared to that in Table 9 of the Clinical Pharmacology Review dated August 9, 2015 (n = 14 versus n = 12).

Compared to the Table 10 in the Clinical Pharmacology Review dated August 9, 2015, the updated table has one fewer subject for the 2 mg/kg dose in ENB-010-10 (n=14 in revised Table 10 versus n=15 in the original Table 10). During the negotiation of the product labeling, the Applicant confirmed that one of the subjects in ENB-010-10 received an asfotase alfa dose of 1 mg/kg, instead of the 2 mg/kg dose described in the Patient Listing of the Clinical Study Report. Therefore, PK results from this subject were not included in the updated Table 10 as presented below.

Note that Table 10 shows the PK parameters used to describe asfotase alfa PK in the product labeling; the data represent results obtained after multiple dosing of asfotase alfa in studies ENB-006-09 and ENB-010-10.

Table 9. Summary of PK parameters following single dosing of asfotase alfa in HPP patients at Week 1 by dose by study (48-hour concentration-time profiles for Study ENB-006-09, 24-hour profiles for Study ENB-009-10, and 48-hour profiles for Study ENB-010-10) (source: Table 2 of Response to Clinical Pharmacology Information Request from August 7, 2015)

	Dose	0.3 mg/kg	0.5 mg/kg	2 mg/kg	2 mg/kg	3 mg/kg
	Study ID	ENB-009-10	ENB-009-10	ENB-006-09	ENB-010-10	ENB-006-09
Parameter	Study Week	Week 1 n=1	Week 1 n=2	Week 1 n=6	Week 1 n=14	Week 1 n=7
Age (year)	Mean ± SD	16.9 ± NA	34.9 ± NA	8.5 ± 2.2	3.3* ± 2.1	9.3 ± 2.3
	Median	16.9	34.9	8.2	3.7	10.1
	Range	(16.9, 16.9)	(15.8, 53.9)	(6.0, 12.5)	(0.1, 6.1)	(6.2, 12.1)
Weight at baseline (kg)	Mean ± SD	52.0 ± NA	65.8 ± NA	21.2 ± 7.9	11.2 ± 5	30.6 ± 16.4
	Median	52.0	65.8	20.6	12.4	27.7
	Range	(52.0, 52.0)	(60.2, 71.3)	(11.4, 35.4)	(3.2, 17.1)	(17.4, 62.6)
t <sub>last</sub> (h)	Mean ± SD	23.92 ± NA	23.99 ± NA	47.92 ± 0.24	48.02 ± 0.11	48.07 ± 0.14
	Median	23.92	23.99	48.00	48.00	48.00
	Range	(23.92, 23.92)	(23.98, 24)	(47.45, 48.1)	(47.75, 48.17)	(47.92, 48.27)
t <sub>max</sub> (h)	Mean ± SD	23.92 ± NA	16.01 ± NA	37.25 ± 8.13	27.17 ± 12.13	34.35 ± 6.02
	Median	23.92	16.01	32.12	27.09	32.12
	Range	(23.92, 23.92)	(16, 16.02)	(31.8, 48.02)	(6.08, 48.15)	(31.93, 48)
C <sub>max</sub> (ng/mL)	Mean ± SD	273 ± NA	487 ± NA	670 ± 142	1175 ± 835	1488 ± 518
	Median	273	487	657	951	1430
	Range	(273, 273)	(428, 545)	(462, 901)	(668, 3990)	(943, 2510)
AUC <sub>t</sub> (h*ng/mL)	Mean ± SD	4390 ± NA	8123 ± NA	23333 ± 6771	45338 ± 30191	53548 ± 17872
	Median	4390	8123	21822	38175	55137
	Range	(4390, 4390)	(7181, 9066)	(15726, 36031)	(25888, 146400)	(34512, 86156)
C <sub>max,nor</sub> (ng/mL/ (mg/kg))	Mean ± SD	910 ± NA	973 ± NA	335 ± 71	588 ± 417	496 ± 173
	Median	910	973	329	476	477
	Range	(910, 910)	(856, 1090)	(231, 451)	(334, 1995)	(314, 837)
AUC <sub>t,nor</sub> (h*ng/mL/ (mg/kg))	Mean ± SD	14635 ± NA	16247 ± NA	11667 ± 3386	22669 ± 15095	17849 ± 5957
	Median	14635	16247	10911	19087	18379
	Range	(14635, 14635)	(14362, 18131)	(7863, 18016)	(12944, 73200)	(11504, 28719)

Source: [Single NCA parameters\\_ENB-010-10\\_48\\_hours.csv.txt](#)

Only data from (b) (4) batch size is presented. Table provided for completeness with PK parameters for ENB-010-10 calculated over the dosing interval of 48 hours vs the requested 32 hours.

t<sub>last</sub>, time of last concentration; t<sub>max</sub>, time of maximal concentration; C<sub>max</sub>, maximal concentration; AUC<sub>t</sub>, area under the concentration-time curves; PK parameters from 48-hour concentration-time profiles for Study ENB-006-09 and Study ENB-010-10, 24-hour profiles for Study ENB-009-10; C<sub>max,nor</sub>, dose normalized C<sub>max</sub>; AUC<sub>t,nor</sub>, dose normalized AUC<sub>t</sub>; \*: Typo in reporting this value in the previous response dated 14 August 2015 (Table 1: Amendment to Clin Pharm IR date 26 Jan 2015.pdf).

Table 10. Summary of PK parameters following multiple dosing of asfotase alfa at Week 3 or Week 6 by dose by study (Source: Table 3 of Response to Clinical Pharmacology Information Request from August 7, 2015)

	Dose	0.3 mg/kg	0.5 mg/kg	2 mg/kg	2 mg/kg	3 mg/kg
	Study ID	ENB-009-10	ENB-009-10	ENB-006-09	ENB-010-10	ENB-006-09
Parameter	Study Week	Week 3 n=6	Week 3 n=6	Week 6 n=6	Week 6 n=14	Week 6 n=6
Age (year)	Mean ± SD	44.5 ± 23.3	49.3 ± 16.5	8.6 ± 2.2	3.4 ± 2.1	9.2 ± 2.6
	Median	52.0	55.9	8.3	3.8	9.5
	Range	(14.9, 66.8)	(15.8, 58)	(6.1, 12.6)	(0.2, 6.2)	(6.3, 12.3)
Weight at baseline (kg)	Mean ± SD	68.2 ± 15.1	71.7 ± 12.7	21.2 ± 7.9	11.2 ± 5	31.1* ± 17.9
	Median	65.7	70.2	20.6	12.4	23.7
	Range	(52.0, 90.7)	(56.8, 88.2)	(11.4, 35.4)	(2.9, 17.1)	(17.4, 62.6)
t <sub>last</sub> (h)	Mean ± SD	24.01 ± 0.02	24.01 ± 0.05	48.02 ± 0.05	48.05 ± 0.11	45.4 ± 6.53
	Median	24.00	24.01	48.01	48.00	48.05
	Range	(23.98, 24.05)	(23.92, 24.08)	(47.97, 48.1)	(47.92, 48.32)	(32.07, 48.12)
t <sub>max</sub> (h)	Mean ± SD	12.00 ± 10.75	8.00 ± 8.31	20.76 ± 10	14.92 ± 10.39	17.34 ± 8.63
	Median	10.00	5.99	18.13	12.00	12.04
	Range	(0, 24.05)	(2.00, 24.05)	(11.92, 32.17)	(0, 32.17)	(12.00, 32.02)
C <sub>max</sub> (ng/mL)	Mean ± SD	1544 ± 1007	3147 ± 1875	2108 ± 788	1794 ± 690	2698 ± 1035
	Median	1340	2360	2080	1555	2690
	Range	(412, 3410)	(1400, 6470)	(905, 3390)	(856, 3510)	(1310, 4240)
AUC <sub>t</sub> (h*ng/mL)	Mean ± SD	24384 ± 8603	56935 ± 32162	89877 ± 33248	66042 ± 25758	111524 ± 52481
	Median	27491	38868	90198	58067	113950
	Range	(8981, 31741)	(31045, 106044)	(37364, 142265)	(27770, 119122)	(51082, 190414)
C <sub>max,nor</sub> (ng/mL/ (mg/kg))	Mean ± SD	5146 ± 3357	6293 ± 3751	1054 ± 394	897 ± 345	899 ± 345
	Median	4467	4720	1040	778	897
	Range	(1373, 11367)	(2800, 12940)	(453, 1695)	(428, 1755)	(437, 1413)
AUC <sub>t,nor</sub> (h*ng/mL/ (mg/kg))	Mean ± SD	81280 ± 28676	113870 ± 64324	44938 ± 16624	33021 ± 12879	37175 ± 17494
	Median	91636	77737	45099	29034	37983
	Range	(29937, 105804)	(62090, 212088)	(18682, 71133)	(13885, 59561)	(17027, 63471)

Source: Multiple NCA parameters.csv.txt

Only data from (b) (4) batch size is presented.

t<sub>last</sub>, time of last concentration; t<sub>max</sub>, time of maximal concentration; C<sub>max</sub>, maximal concentration; AUC<sub>t</sub>, area under the concentration-time curves over the dosing interval; PK parameters from 48-hour profiles for Studies ENB-006-09 and ENB-010-10 and 24-hour profiles for Study ENB-009-10; C<sub>max,nor</sub>, dose normalized C<sub>max</sub>; AUC<sub>t,nor</sub>, dose normalized AUC<sub>t</sub>; \*:

Typo in reporting this value in the previous response dated 14 August 2015 (Table 1: Amendment to Clin Pharm IR date 26 Jan 2015.pdf).

Table 13. Summary of PK parameters following multiple dosing of asfotase alfa in Study ENB-009-10 (Source: Table 4 of Response to Clinical Pharmacology Information Request from August 7, 2015)

Parameter	Dose	0.3 mg/kg			0.5 mg/kg		
	Study Week	Week 3 n=6	Week 12 n=6	Week 24 n=4	Week 3 n=6	Week 12 n=5	Week 24 n=4
Age (year)	Mean ± SD	44.5 ± 23.3	44.7 ± 23.3	48.9 ± 23	49.3 ± 16.5	48.1 ± 18.1	56.5 ± 1.6
	Median	52.0	52.2	55.5	55.9	56.0	56.5
	Range	(14.9, 66.8)	(15.1, 67)	(17.4, 67.2)	(15.8, 58)	(15.9, 58.2)	(54.4, 58.4)
Weight at baseline (kg)	Mean ± SD	68.2 ± 15.1	68.2 ± 15.1	68.5 ± 17.3	71.7 ± 12.7	72.2 ± 14.1	75.2 ± 14.3
	Median	65.7	65.7	65.7	70.2	71.3	78.0
	Range	(52.0, 90.7)	(52.0, 90.7)	(52.0, 90.7)	(56.8, 88.2)	(56.8, 88.2)	(56.8, 88.2)
t <sub>last</sub> (h)	Mean ± SD	24.01 ± 0.02	24.02 ± 0.09	24.05 ± 0.08	24.01 ± 0.05	24 ± 0.07	24.06 ± 0.06
	Median	24.00	24.02	24.01	24.01	24.00	24.05
	Range	(23.98, 24.05)	(23.88, 24.17)	(24.00, 24.17)	(23.92, 24.08)	(23.9, 24.08)	(24.00, 24.12)
t <sub>max</sub> (h)	Mean ± SD	12.00 ± 10.75	9.68 ± 11.13	6.03 ± 6.71	8.00 ± 8.31	14.03 ± 9.82	8.52 ± 10.93
	Median	10.00	3.09	3.07	5.99	12.00	4.99
	Range	(0, 24.05)	(1.87, 24.03)	(2.00, 16.00)	(2.00, 24.05)	(2.00, 24.08)	(0, 24.1)
C <sub>max</sub> (ng/mL)	Mean ± SD	1544 ± 1007	1210 ± 494	969 ± 284	3147 ± 1875	2380 ± 734	2410 ± 1239
	Median	1340	1235	933	2360	2250	2430
	Range	(412, 3410)	(616, 1780)	(711, 1300)	(1400, 6470)	(1530, 3560)	(1140, 3640)
AUC <sub>t</sub> (h*ng/mL)	Mean ± SD	24384 ± 8603	25979 ± 12068	20699 ± 6563	56935 ± 32162	50483 ± 17526	51276 ± 29066
	Median	27491	25540	19204	38868	49890	49552
	Range	(8981, 31741)	(10585, 40307)	(15378, 29009)	(31045, 106044)	(28942, 76916)	(23542, 82456)
C <sub>max,nor</sub> (ng/mL/ (mg/kg))	Mean ± SD	5146 ± 3357	4032 ± 1646	3230 ± 946	6293 ± 3751	4760 ± 1468	4820 ± 2478
	Median	4467	4117	3108	4720	4500	4860
	Range	(1373, 11367)	(2053, 5933)	(2370, 4333)	(2800, 12940)	(3060, 7120)	(2280, 7280)
AUC <sub>t,nor</sub> (h*ng/mL/ (mg/kg))	Mean ± SD	81280 ± 28676	86596 ± 40226	68996 ± 21875	113870 ± 64324	100966 ± 35052	102551 ± 58133
	Median	91636	85134	64014	77737	99780	99105
	Range	(29937, 105804)	(35285, 134358)	(51260, 96697)	(62090, 212088)	(57884, 153832)	(47084, 164912)

Source: [Multiple NCA parameters.csv.txt](#)

Only data from (b) (4) batch size is presented.

t<sub>last</sub>, time of last concentration; t<sub>max</sub>, time of maximal concentration; C<sub>max</sub>, maximal concentration; AUC<sub>t</sub>, area under the concentration-time curves over the dosing interval;

C<sub>max,nor</sub>, dose normalized C<sub>max</sub>; AUC<sub>t,nor</sub>, dose normalized AUC<sub>t</sub>

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/s/  
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CHRISTINE Y HON  
10/23/2015

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10/23/2015

## CLINICAL PHARMACOLOGY INDIVIDUAL STUDY REVIEW

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BLA	<b>125513 (eCTD sequence 0000)</b>
Drug Name	<b>Asfotase Alfa (Strensiq)</b>
Formulation	<b>Sterile aqueous solution for subcutaneous (SC) injection</b>
Formulation Strength	<b>40 mg/mL and 100 mg/mL</b>
Dosing Regimen	<b>1 mg/kg SC 6 times/weeks or 2 mg/kg SC 3 times/week</b>
Route of Administration	<b>Subcutaneous</b>
Description of Submission	<b>Original NME BLA</b>
Received Date	<b>12/23/2014</b>
PDUFA Date	<b>11/23/2015</b>
Clinical Pharmacology Reviewer	<b>Christine Yuen-Yi Hon, Pharm.D</b>
Clinical Pharmacology Team Lead	<b>Yow-Ming Wang, Ph.D.</b>
OCP Division	<b>DCP3</b>
OND Division	<b>DGIEP</b>
Applicant	<b>Alexion</b>
Proposed Indication	<b>Treatment of Patients with Infantile-Onset and Juvenile-Onset Hypophosphatasia</b>

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This review provides the Individual Study Review (ISR) for clinical studies ENB-001-08, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, ENB-009-10, and ENB-010-10. Because of the small number of subjects with different disease phenotypes in some of the studies and the use of different dosage regimens and dose adjustments of asfotase alfa in all studies, it is most helpful to summarize the clinical pharmacology results using an integrated approach, which were presented in the Clinical Pharmacology Review dated August 9, 2015. Therefore, this ISR primarily focuses on the individual study design. The readers are referred to the Clinical Pharmacology Review dated August 9, 2015 for details about the results of the pharmacokinetic (PK), pharmacodynamics (PD)/efficacy, pharmacogenomic, immunogenicity, and safety data.

## ENB-001-08

ENB-001-08 was a 1-month, multicenter, open-label, dose-escalating, first-in-human (FIH) study of asfotase alfa in adults with HPP.

### Objectives

#### Primary

To assess safety and tolerability of asfotase alfa given intravenously (IV) as a single dose and given subcutaneously (SC) in 3 weekly doses

#### Secondary

To assess pharmacokinetics (PK) and bioavailability of ENB-0040 given IV and SC

### Methodology

There were two cohorts of patients in this study. Cohort 1 (n=3) received asfotase alfa 3 mg/kg IV the first week followed by 3 doses at 1 mg/kg SC at weekly (QW) intervals from weeks 2 to 4. Cohort 2 (n=3) received asfotase alfa 3 mg/kg IV the first week followed by 3 doses at 2 mg/kg SC at weekly intervals from weeks 2 to 4. Table 1 summarizes the dosing and assessment schedules for Study ENB-001-08.

Table 1. Study ENB-001-08 Dosing and Assessment Schedules (Source: Table 4 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Dosing and Sampling Schedules <sup>a</sup>	
Patient Group	Adults
Dosing Regimen	3 mg/kg IV x 1; 1 or 2 mg/kg SC x 3; weekly dosing
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	pre-dose, during, and at end of infusion, then 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 hours after IV infusion; pre-dose, 6, 12, 24, 48, 72 hours after first SC injection; pre-dose and 24 hours after second SC injection; pre-dose, 6, 12, 24, 48, 72, 168 hours after third SC injection; week 8
PLP	Screening, baseline, and day 25
PPi	Baseline, days 1, 2, 8, 15, 22, and 25
RGI-C	Not Applicable
RSS	Not Applicable
BOT-2	Not Applicable
6MWT	Not Applicable
Anti-asfotase alfa Antibody Testing	Pre-dose, days 1 and 25; week 8

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-001-08 clinical study protocol](#).

### Results

Six Caucasians subjects were enrolled in the study, 4 were female and 2 were male. The mean age and weight ( $\pm$  standard deviation [SD]) were 44.8 (12.6) years old and 70.6 (16.5) kg, respectively. Four of these six subjects subsequently enrolled in Study ENB-009-10.

### PK

The PK results from ENB-001-08 can be found in Section 2.5.2 of the Clinical Pharmacology Review dated August 9, 2015.

Safety Including Immunogenicity

Asfotase alfa was generally well tolerated in all subjects. Two subjects tested positive for anti-asfotase alfa antibody (ADA+) with a rate of 33% (2/6). However, none tested positive for neutralizing antibodies (Nab+).

## Study ENB-002-08

Study ENB-002-08 was a 24-week, international, multicenter, open-label study to assess the safety, tolerability, and pharmacology of asfotase alfa in severely affected infants and children  $\leq$  36 months old with onset of HPP signs prior to 6 months of age.

### Objectives

#### Primary

- To determine the efficacy of asfotase alfa in treating the skeletal manifestations of infantile HPP
- To determine the safety and tolerability of asfotase alfa given IV in a single dose and SC in repeat doses

#### Secondary

- To evaluate the PK of asfotase alfa given IV as a single dose and SC in repeated doses
- To evaluate the bioavailability of SC asfotase alfa

### Methodology

Subjects received a single IV asfotase alfa infusion at a dose of 2 mg/kg followed by 3 times weekly SC injections of 1 mg/kg with dose adjustments allowed to optimize response for 24 weeks. The dose adjustments were allowed to start after one month of treatment; the SC dose could be increased to 2 mg/kg for lack of efficacy defined as 2 of the 3 following outcomes:

- Failure to show radiographic improvement in rickets.
- Deterioration of pulmonary function.
- Worsening of failure to thrive

After 3 months of treatment, if the subject had lack of efficacy by:

- One of the 3 outcomes stated above, the SC dose could be increased to 2 mg/kg
- Two of the 3 outcomes stated above, the SC dose could be increased to 3 mg/kg

Table 2 summarizes the dosing and assessment schedules for Study ENB-002-08.

### Results

Eleven subjects with either perinatal- or infantile-onset HPP were enrolled; 10 subjects completed the study. The mean patient age ( $\pm$  SD) was 58.8 (59.2) weeks old.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

Table 2: Study ENB-002-08 Dosing and Assessment Schedules (Source: Table 6 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

<b>Dosing and Sampling Schedules<sup>a</sup></b>	
Patient Group	Infants & young children
Dosing Regimen	2 mg/kg IV; after 1 week, 1 mg/kg SC three times/week; after one month, 2 mg/kg three times per week, after three months, dose adjustments could be made up to 3 mg/kg three times per week
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	pre-dose and 1, 4, 12, 24, 48, and 96 hours after IV infusion; pre-dose and 6, 12, 18, 24, 36, and 48 hours after SC doses; pre-dose only after second through fifth SC doses; pre-dose and 6, 12, 18, 24, 36, and 48 hours after the sixth SC dose or subsequent dose as soon as was practicable. An additional PK sample may have also been collected 72 hours after the sixth (or subsequent) dose if there were 3 days between doses. pre-dose at weeks 4, 12, 18, and 24
PLP	Screening and weeks 12 and 24
PPi	Day 1 of week 1, day 8 of week 2, and weeks 12, 18, and 24
RGI-C	Screening (or Baseline) visit and weeks 4, 12, and 24
RSS	Screening (or Baseline) visit and weeks 4, 12, and 24
BOT-2	Not Applicable
6MWT	Not Applicable
Anti-Asfotase Alfa Antibody Testing	Pre-dose and day1 of week 1, weeks 4, 8, 12, 18, and 24

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-002-08 clinical study protocol](#).

## ENB-003-08

Study ENB-003-08 was to collect long-term safety and efficacy data in patients successfully completing clinical trial ENB-002-08.

### Objectives

#### Primary

- To determine the long-term tolerability of SC asfotase alfa
- To determine the long-term efficacy of asfotase alfa in treating rickets in infants and young children with HPP

#### Secondary

- To evaluate the long-term PD of SC asfotase alfa
- To evaluate the effect of SC asfotase alfa on growth and development, mortality, and other clinical signs and symptoms of HPP in infants and young children

### Methodology

Subjects who completed ENB-002-08 and met study eligibility criteria were enrolled in this study to receive up to 84 months of treatment (including the first 6 months). Table 3 summarizes the dosing and assessment schedules for Study ENB-003-08.

Table 3. Study ENB-003-08 Dosing Assessment Schedules (Source: Table 7 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Dosing and Sampling Schedules <sup>a</sup>	
Patient Group	Infants & young children
Dosing Regimen	SC injections 3times per week at final dose received in ENB-002-08, dose adjustments allowed and max dose = 40mg
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	Not Applicable
PLP	Baseline and months 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 54, 60
PPi	Baseline and months 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 54, 60
RGI-C	Baseline and months 3, 6, 9, 12, 18, 24, 30, 36, 48, 60
RSS	Baseline and months 3, 6, 9, 12, 18, 24, 30, 36, 48, 60
BOT-2	Baseline and months 6, 12, 18, 24, 30, 36, 42, 48, 54, 60
6MWT	Not Applicable
Anti-Asfotase Alfa Antibody Testing	Baseline and months 3, 6, 9, 12, 18, 24, 30, 36, 42, 48, 54, 60

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-003-08 clinical study protocol](#).

### Results

Ten of the 11 patients who enrolled in ENB-002-08 successfully completed the clinical trial and participated in extension study. Parental consent was withdrawn in association with an infusion-associated reaction in ENB-002-08 for the remaining subject. As of the analysis cutoff date of October 29, 2014, 9 patients had completed a minimum of 54 months of study drug treatment; 4

of those 9 patients had completed over 60 months of study drug treatment. Study ENB-003-08 is currently ongoing.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

## ENB-006-09

Study ENB-006-09 was a 24-week, international, multicenter, open-label, historical control study to assess the safety, efficacy, PK, and PD of asfotase alfa in children with HPP between the ages of 5 and 12 years.

### Objectives

#### Primary

- To assess the changes in skeletal radiographs of the wrists and knees as compared with historical controls using a qualitative Clinical Global Impression of Change (CGI-C) scoring system
- To determine the safety and tolerability of asfotase alfa SC thrice weekly

#### Secondary

- To assess the change in osteomalacia (as measured by transiliac crest bone biopsy), height (Z-scores), and plasma inorganic pyrophosphate (PPi) and pyridoxal-5'-phosphate (PLP)
- To assess the PK of asfotase alfa
- To compare the safety, PK, and PD of two doses of asfotase alfa

### Methodology

Patients were required to have open growth plates and the presence of HPP-related rickets on skeletal radiographs of the wrists and knees to be eligible for inclusion in ENB-006-09. Patients were randomized to receive SC injections of 2 or 3 mg/kg asfotase alfa (a total of 6 or 9 mg/kg/week) 3 times weekly for 24 weeks. Table 4 summarizes the dosing and assessment schedules for Study ENB-006-09.

Table 4: Study ENB-006-09 Dosing and Assessment Schedules (Source: Table 8 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Dosing and Sampling Schedules <sup>a</sup>	
Patient Group	Children & early adolescents
Dosing Regimen	2 or 3 mg/kg SC three times per week
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	Pre-dose and at 6, 12, 24, 32, and 48 hours post-dose at baseline and week 6 and pre-dose only at weeks 12, 18, and 24
PLP	Baseline and only pre-dose on weeks 6 and 12; week 24
PPi	Baseline and only pre-dose on weeks 6 and 12; week 24
RGI-C	Screening, and pre-dose on weeks 6, 12, and 24
RSS	Screening, and pre-dose on weeks 6, 12, and 24
6MWT	Screening, baseline, and pre-dose on weeks 12 and 24
BOT-2	Screening, baseline, and pre-dose on weeks 12 and 24
Anti-Asfotase Alfa Antibody Testing	Pre-dose at baseline; pre-dose on weeks 6, 12; weeks 18, 24

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-006-09 clinical study protocol](#).

## **Results**

Thirteen subjects were enrolled; 5 had infantile-onset and 8 had juvenile-onset HPP. The mean patient age ( $\pm$  SD) was 8.8 (2.2) years old.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

## **ENB-008-10**

Study ENB-008-10 is an ongoing, open-label extension study of asfotase alfa in 12 patients who previously received treatment under clinical study ENB-006-09 and completed the study.

### **Objectives**

#### Primary

- To assess the long-term tolerability of SC asfotase alfa
- To assess the proportion of asfotase alfa-treated patients showing radiographic change in rickets severity (as assessed by skeletal radiographs of the hands/wrists and knees) from the Baseline of ENB-006-09 relative to the End of Study visit in ENB-008-10 using the RGI-C scale score

#### Secondary

- To evaluate the long-term PK of SC asfotase alfa
- To evaluate the effect of SC asfotase alfa on reduction in PPi and PLP
- To assess the effect of SC asfotase alfa on height (Z-scores), Six-Minute Walk Test (6MWT), Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2), hand-held dynamometry (HHD), body mass index (BMI) and arm span (Z-scores), dual energy X-ray absorptiometry (DEXA), Pediatric Orthopedic Society of North America (POSNA) Pediatric Outcomes Data Collection Instrument (PODCI), and Child Health Assessment Questionnaire (CHAQ)

### **Methodology**

Patients who completed Study ENB-006-09 and met the eligibility criteria were enrolled to continue asfotase alfa treatment under ENB-008-10. All patients received the same amount of asfotase alfa each week, either 0.5 mg/kg 6 times per week or 1 mg/kg 3 times per week (a total of 3 mg/kg/week) according to the initial protocol. The dosing was changed to 1 mg/kg 6 times per week or 2 mg/kg 3 times per week (a total of 6 mg/kg/week) in Protocol Amendment 4 dated February 1 2011. Study ENB-008-10 includes an additional 42 months of asfotase alfa treatment. Table 5 summarizes the dosing and assessment schedules for Study ENB-008-10.

### **Results**

Twelve of 13 subjects in ENB-006-09 enrolled in the extension study ENB-008-10. The mean age ( $\pm$  SD) was 8.8 (2.2) years old. As of the data analysis cut-off date of November 5, 2014, all patients treated with asfotase alfa have completed at least 4 years (48 months) of treatment with asfotase alfa. Study ENB-008-10 is currently ongoing.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

Table 5: Study ENB-008-10 Dosing and Assessment Schedules (Source: Table 9 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

<b>Dosing and Sampling Schedules<sup>a</sup></b>	
Patient Group	Children & early adolescents
Dosing Regimen	1 mg/kg SC 6 times per week or 2 mg/kg 3 times per week SC
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	pre-dose at base-line, and months 3, 6, 9, and q6 months thereafter
PLP	Baseline, and months 3, 6, 9, and q6 months thereafter
PPi	Baseline, and months 3, 6, 9, and q6 months thereafter
RGI-C	Baseline, and months 3, 6, 9, and q6 months thereafter
RSS	Baseline, and months 3, 6, 9, and q6 months thereafter
6MWT	Baseline, and months 3, 6, 9, and q6 months thereafter
BOT-2	Baseline, and months 3, 6, 9, and q6 months thereafter
Anti-Asfotase Alfa Antibody Testing	Baseline, and months 3, 6, 9, and q6 months thereafter

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-008-10 clinical study protocol](#).

## **ENB-009-10**

Study ENB-009-10 is a multicenter, open-label, concurrent control study to assess the safety, tolerability, and pharmacology of asfotase alfa in adolescent and adult patients ages 13 to 66 years.

### **Objectives**

#### Primary

- To evaluate the effect of asfotase alfa on reduction in plasma PPi and plasma PLP
- To assess the tolerability of daily SC injections of asfotase alfa

#### Secondary

- To evaluate the change in HPP-related osteomalacia as measured by transiliac crest bone biopsy, change in DEXA, and change in 6MWT

### **Methodology**

Patients were required to have a pre-established clinical diagnosis of HPP, with evidence of osteomalacia on bone biopsy. Patients were initially randomized to 1 of 3 treatment groups (placebo control, 0.3 mg/kg/day asfotase alfa, or 0.5 mg/kg/day asfotase alfa) for a 24-week duration (primary treatment period; PTP). Upon completion of the PTP, all patients were eligible to continue in the open-label extension treatment period (ETP) of this study, where all patients received treatment with asfotase alfa 0.5 mg/kg/day for approximately 24 weeks. Thereafter, the dose was increased to 1 mg/kg 6 times per week. Due to the timing for implementation of protocol amendments, some patients received the 3.5 mg/kg/week dosage regimen for 24 to 48 weeks.

Dose adjustments could be made per protocol every 3 months based upon changes in weight in order to maintain the weekly target dose. The maximum daily dose of asfotase alfa was not to exceed 80 mg during the PTP or ETP unless otherwise specified by the Investigator and Medical Monitor prior to implementation. Table 6 summarizes the dosing and assessment schedules for Study ENB-009-10.

### **Results**

Nineteen patients were enrolled; 3 (15.8%) patients withdrew from the study. One subject withdrew (after Week 96) due to injection site reaction, 1 withdrew due to poor compliance (after Week 120), and 1 withdrew due to injection site reactions (lipohypertrophy) (after Week 170). The mean age at enrollment was 40.9 years at Baseline, but the median age of the control group was much lower than the combined asfotase alfa treatment group (21.0 years [range: 13, 58] vs 55 years [range: 14, 66], respectively). The majority of patients were white (94.7%), female (63.2%) adults aged  $\geq 18$  years (68.4%). Twelve of the 19 subjects (63.2%) had juvenile-onset HPP; 4 patients had infantile-onset HPP; and 2 had adult-onset HPP.

All of the patients originally randomized to asfotase alfa treatment (n = 13) during the PTP had received at least 96 weeks of exposure to asfotase alfa as of data cutoff date November 5, 2014;

12 had at least 144 weeks of treatment with asfotase alfa (1 asfotase alfa patient had withdrawn from the study), and 10 had 192 weeks of exposure (1 more asfotase alfa patient had withdrawn and 1 patient had not reached the Week 192 visit prior to data cut-off). Of the 6 patients originally assigned to the control group during the PTP, all had received at least 96 weeks of exposure to asfotase alfa during the ETP and 5 had received 144 weeks of exposure to asfotase alfa during the ETP (1 patient had withdrawn after 96 weeks of exposure). Study ENB-009-10 is currently ongoing.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

Table 6: Study ENB-009-10 Dosing and Assessment Schedules (Source: Table 10 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

<b>Dosing and Sampling Schedules<sup>a</sup></b>	
Patient Group	Adolescents and Adults
Dosing Regimen	0.3 mg/kg/day SC, 0.5 mg/kg/day SC, or no treatment first 24 weeks; 0.5 mg/kg/day SC for 24 additional weeks; 1 mg/kg/day, 6 days/week for an additional 48 weeks
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	Baseline; pre-dose and 2, 4, 8, 12, 16, and 24 hours post-dose on weeks 3, 12, and 24; Pre-dose sample on week 6
PLP	Screening, baseline and Weeks 6, 12, 24, 30 (control group only), 36, 48, and q6M thereafter
PPi	Baseline and weeks 6, 12, 24, 30 (control group only), 36, 48, and q6M thereafter
RGI-C	Screening and Weeks 24 and 48 and q6M thereafter
RSS	Screening and Weeks 24 and 48 and q6M thereafter
6MWT	Screening, baseline and Weeks 12, 24, 36 (control group only), 48 and q6M thereafter
BOT-2	Screening, baseline and Weeks 12, 24, 36 (control group only), 48 and q6M thereafter
Anti-Asfotase Alfa Antibody Testing	pre-dose at baseline, weeks 3, 6, 12, 24, 27 (control group only), 30 (control group only), 36, 48, and q6M thereafter

<sup>a</sup> The dosing and sampling schedules are taken from ENB-009-10 clinical study protocol.

## **ENB-010-10**

Study ENB-010-10 is a multicenter, open-label, study of the safety, efficacy, and PK of asfotase alfa in infants and children up to and including 5 years of age with HPP patients.

### **Objectives**

#### Primary

- To determine the effect of asfotase alfa treatment on skeletal manifestations of HPP as measured by RGI-C scale
- To evaluate safety and tolerability of repeated SC injections of asfotase alfa

#### Secondary

- To evaluate the percentage of patients who are not mechanically ventilated at the time of enrollment, but are alive and ventilator-free after receiving asfotase alfa as compared to an age-matched historical control group
- To assess the effect of asfotase alfa treatment on respiratory function, physical growth, and tooth loss
- To evaluate the PK properties of asfotase alfa
- To assess the effect of asfotase alfa on plasma PPi and plasma PLP

### **Methodology**

Patients were required to have perinatal/infantile-onset HPP, defined as onset of HPP signs/symptoms prior to 6 months of age. Patients received a total of 6 mg/kg/week of asfotase alfa administered by SC injection, either 1 mg/kg asfotase alfa 6 times per week or 2 mg/kg asfotase alfa 3 times per week. Dose adjustments could be made for changes in weight and/or to improve safety and efficacy. Table 7 summarizes dosing and assessment schedules for ENB-010-10.

### **Results**

As of the November 22, 2013 data cutoff date, 28 patients were enrolled, with a mean age ( $\pm$ SD) of 118 (113) weeks old. These infantile-onset HPP patients, together with those in ENB-002-08, were included in the overall survival analysis in the Clinical Pharmacology Review dated August 9, 2015.

As of the November 12, 2014 data cutoff date, an additional 31 patients were enrolled in the study since the last data cut-off date; exposure for these patients ranges from less than 3 to approximately 12 months. Study ENB-010-10 is currently ongoing.

Please see Clinical Pharmacology Review dated August 9, 2015 for details about the results of the PK, PD/efficacy, immunogenicity, and safety data.

Table 7: Study ENB-010-10 Dosing and Assessment Schedules (Source: Table 11 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Dosing and Sampling Schedules <sup>a</sup>	
Patient Group	Infants and children ≤5
Dosing Regimen	1 mg/kg/day for 6 days/week or 2 mg/kg/day for 3 days/week (max dose = 40 mg)
Asfotase Alfa Concentration Measured as Activity (pharmacokinetics assay)	Baseline and Week 6, pre-dose, 6, 12, 24, 32, and 48 hours post-dose; Pre-dose on months 3, 6, 9, 12, 15, 18, 24,30, 32, 36, 42, 48
PLP	Screening, baseline, week 6, and months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48
PPi	Baseline, week 6, and months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48
RGI-C	Screening, months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48
RSS	Screening, months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48
GMWT	Not Applicable
BOT-2	Baseline, months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48
Anti-Asfotase Alfa Antibody Testing	Pre-dose at baseline, weeks 3 and 6, and months 3, 6, 9, 12, 15, 18, 24, 30, 36,42, and 48

<sup>a</sup> The dosing and sampling schedules are taken from [ENB-010-10 clinical study protocol](#).

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/s/  
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CHRISTINE Y HON  
10/23/2015

YOW-MING C WANG  
10/23/2015

## CLINICAL PHARMACOLOGY REVIEW

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BLA	<b>125513 (eCTD sequence 0000)</b>
Drug Name	<b>Asfotase Alfa (Strensiq)</b>
Formulation	<b>Sterile aqueous solution for subcutaneous (SC) injection</b>
Formulation Strength	<b>40 mg/mL and 100 mg/mL</b>
Dosing Regimen	<b>1 mg/kg SC 6 times/weeks or 2 mg/kg SC 3 times/week</b>
Route of Administration	<b>Subcutaneous</b>
Description of Submission	<b>Original NME BLA</b>
Received Date	<b>12/23/2014</b>
PDUFA Date	<b>11/23/2015</b>
Clinical Pharmacology Reviewer	<b>Christine Yuen-Yi Hon, Pharm.D</b>
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Genomics and Targeted Therapy Reviewer	<b>Sarah Dorff, Ph.D.</b>
Genomics and Targeted Therapy Team Lead	<b>Christian Grimstein, Ph.D.</b>
OCP Division	<b>DCP3</b>
OND Division	<b>DGIEP</b>
Applicant	<b>Alexion</b>
Proposed Indication	<b>Treatment of Patients with Infantile-Onset and Juvenile-Onset Hypophosphatasia</b>

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## 1. EXECUTIVE SUMMARY

Hypophosphatasia (HPP) is a rare genetic disorder caused by the loss-of-function mutation(s) in the gene encoding tissue-nonspecific alkaline phosphatase (TNSALP). HPP is characterized by defective bone mineralization and impaired phosphate and calcium regulation, resulting in elevations of several TNSALP substrates including inorganic pyrophosphate (PPi) and pyridoxal-5'-phosphate (PLP). Subsequently, the disease leads to progressive damage to vital organs along with other clinical sequelae including deformity and destruction of bones, pain and profound muscle weakness, respiratory failure, seizures, impaired renal function, impaired mobility, and dental abnormalities.

Asfotase alfa is a soluble fusion glycoprotein comprised of two identical polypeptide chains, each of which contains the catalytic domain of human TNSALP, the human immunoglobulin (Ig) G1 Fc domain, and a deca-aspartate peptide domain (D10) used for bone-targeting. Asfotase alfa is a bone targeted enzyme replacement therapy (ERT) designed to address the underlying cause of HPP by normalizing the genetically defective metabolic process and aim to prevent or reverse the complications of dysregulated mineral metabolism.

The proposed indication for asfotase alfa is (b) (4) in patients with infantile- and juvenile-onset HPP. The proposed dosing regimen is 6 mg/kg/week to be administered either as 2 mg/kg SC three times per week (3x/week) or 1 mg/kg SC six times per week (6x/week).

Asfotase alfa was granted Orphan Drug Designation on September 12, 2008 and Fast Track Designation on May 14, 2009 for the treatment of HPP. It was granted Breakthrough Therapy designation on May 21, 2013 for the treatment of HPP in perinatal-, infantile-, and juvenile onset phenotypes. Perinatal and infantile HPP include patients with onset of first signs/symptoms at < 6 months of age, and juvenile HPP consists of patients whose onset is  $\geq$  6 months and  $\leq$  18 years of age.

### 1.1 Recommendation

From a clinical pharmacology perspective, the information submitted in this BLA is acceptable to support the approval of asfotase alfa for the proposed indication.

We recommend the use of 40 mg/mL, not 100 mg/mL, formulation strength for infants and young children with infantile-onset HPP because the exposure achieved with 100 mg/mL formulation was estimated to be 24% lower than the exposure achieved with 40 mg/mL formulation.

(b) (4) is a major product quality attribute that affects the systemic exposure of asfotase alfa; (b) (4)  
We recommend (b) (4)  
(b) (4) a more consistent exposure, and to achieve the desirable asfotase alfa exposure identified by the exposure-response relationships observed in clinical studies. (b) (4)

## 1.2 Phase 4 Requirements/Commitments

No post-marketing requirements or commitments are recommended for this submission.

## 1.3 Summary of Clinical Pharmacology Findings

### Pharmacokinetics (PK)

Asfotase alfa PK parameter values after SC administration were available in 38 HPP patients who received daily dose of 0.3 mg/kg and 0.5 mg/kg or a three-times-per-week dosing of 2 mg/kg and 3 mg/kg. Twenty two of these patients had infantile-onset HPP, 14 had juvenile-onset HPP, one had adult-onset HPP, and the remaining patient had unknown disease onset status. The mean age of the patients was 18.1, with a range of 1 month to 66 years old. The mean weight at baseline was  $34.1 \pm 27.9$  kg.

After multiple dosing of 2 mg/kg SC 3x/week for 6 weeks, the mean time to maximum concentration ( $t_{max}$ ) ranged from 15 to 20 hours ( $n = 21$ ). The mean maximum concentration ( $C_{max}$ ) ranged from 1576 to 1781 U/L, and the mean area under the concentration-time curve over the dosing interval ( $AUC_t$ ) at Week 6 ranged from 58743 to 75985 h\*U/L.

Asfotase alfa PK exhibits dose proportionality across the dose range of 0.3 mg/kg to 3 mg/kg and appears to be time-independent. The  $C_{max}$  and  $AUC_t$  values after multiple dosing were higher than the values after the first dose, with accumulation ratios ranging from 5 to 6 for daily dosing at 0.3 to 0.5 mg/kg and from 2.5 to 4 for three-times-per-week dosing at 2 to 3 mg/kg, which is consistent with the observed elimination half-life of approximately 130 hours after intravenous (IV) dosing. Steady state exposure was achieved as early as three weeks following initial dose administration. When administered at 2 mg/kg 3x/week, asfotase alfa PK at Week 6 appeared similar between patients in two age groups (mean age of 3.1 and 7.8 years old).

Asfotase alfa concentration-time profiles following SC administration in HPP patients were well described by a linear, 2-compartment, first-order absorption pharmacokinetic model used for population PK (Pop-PK) analysis. The typical value of clearance (CL) was 12.7 L/day for a subject who has a body weight of 70 kg, is negative for antidrug antibodies (ADA-), and receives a product with (b) (4). The inter-subject variability of CL was 46.5%. The central volume of distribution ( $V_2$ ) was 4.55 L and the peripheral volume of distribution ( $V_3$ ) was 44.6 (32.1 – 62.0) L, indicating that asfotase alfa is initially distributed in the intra-vascular space and then distributes to the extra-vascular space. The inter-subject variability for  $V_2$  and  $V_3$  was 85.5% and 42.7%, respectively. The estimated absolute bioavailability was 62% following SC administration of the (b) (4) scale product in 40 mg/mL.

Body weight was a significant covariate for the PK parameters  $V_2$ ,  $V_3$ , and CL; asfotase alfa exposure increased with body weight. Immunogenicity was a covariate for CL; formation of antidrug antibodies (ADA) was associated with a higher CL. Compared to in the absence of ADA (ADA-), the CL value was 11% higher in the presence of ADA without neutralizing capability

(ADA+/NAb-) and 21% higher in the presence of ADA with neutralizing capability (ADA+/NAb+).

### Exposure-Response (E-R) Relationships

For the infantile-onset HPP patients, asfotase alfa treatment is associated with an increase in overall survival in an exposure-dependent manner.

The E-R for growth in infantile- and juvenile-onset HPP patients was not apparent, as there was no apparent correlation between individual average concentration over the entire study period ( $C_{avg}$ ) and the slope of Z-score for height.

For juvenile-onset HPP patients, an E-R relationship was observed between estimated average asfotase alfa concentration at steady state ( $C_{avg,ss}$ ) and multiple pharmacodynamic (PD) measurements, including the Bruininks-Oserestsky Test of Motor Proficiency, Second Edition (BOT-2) score, 6 Minute Walk Test (6MWT), Radiologic Global Impression of Change (RGI-C) and Rickets Severity Scale (RSS) scores, and plasma PPI and PLP concentrations. These E-R curves showed that response rapidly improved at low concentrations, followed by a more gradual improvement with increasing concentration until a plateau was reached at a  $C_{avg,ss}$  concentration of approximately 1500 - 2000 U/L.

The proposed dosing regimen is 6 mg/kg/week to be administered either as 2 mg/kg SC 3x/week or 1 mg/kg SC 6x/week. The available E-R relationships for effectiveness of asfotase alfa support this proposed regimen. Across patients with different weights, the mean values of the estimated  $C_{avg}$  from the proposed dosing regimen were generally above 2000 U/L which was at or above the beginning of the plateau of the E-R relationship for effectiveness. The exposures associated with the proposed 6 mg/kg weekly regimen range from 1430 to 2930 U/L.

### PK Comparability between Products with Different Batch Size and Formulation Strength

In addition to the to-be-marketed products manufactured at (b) (4) scale, a (b) (4) scale product was also used in the clinical trials. The PK of these two products are most likely comparable. Available PK data showed that (b) (4) product had a 2-fold lower exposure than (b) (4) product, based on an intra-subject comparison of the PK exposure in four subjects who received both products at three different study visits. (b) (4)

The clinical development program used two formulation strengths, 40 mg/mL and 100 mg/mL; 40 mg/mL was used in younger children with lower body weight and 100 mg/mL was used in older children with higher body weight. The PK comparability between the 40 mg/mL and 100 mg/mL formulation strength products could not be concluded. Based on the PK data following the first SC dose administration of asfotase alfa in 35 HPP patients, the least-squares mean ratios for normalized  $C_{max}$  ( $C_{max,nor}$ ) and  $AUC_{t,nor}$  for the 100 mg/mL formulation strength were 89.9 % (90% CI: (b) (4)) and 116 % (90% CI: (b) (4)), respectively, with the 40 mg/mL formulation strength as the reference product. Furthermore, results of Pop-PK analysis indicated

that the 100 mg/mL formulation strength achieved a lower exposure (b) (4)

Due to the lower exposure with 100 mg/mL formulation strength and the lack of clinical experience with 100 mg/mL strength product in pediatric infantile-onset HPP patients to inform its efficacy with respect to overall survival, only the 40 mg/mL formulation strength should be used in pediatric infantile-onset HPP patients.

Effects of (b) (4) on PK and Recommendation for (b) (4) Specification

(b) (4)

### Immunogenicity

Among 71 subjects with post baseline immunogenicity data, 80% (59) subjects were ADA+. Of these ADA+ patients, 54% remained ADA+ later in the study while 46% had at least one ADA- sample. A few patients had ADA titer values >500 for a prolonged period, but low ADA titers were observed in the majority of the patients. Among the 57 subjects with ADA, 25 (44%) subjects were positive for neutralizing antibodies (NAb+) and 32 (56%) subjects were NAb-.

Immunogenicity had a negative impact on the PK of asfotase alfa. Asfotase alfa exposure in ADA- subjects was approximately 1.5- to 2-fold greater than the exposure in ADA+ subjects, based on a subset of 31 pediatric patients with HPP who have 48-hour AUC data available at Week 6. This difference in the observed exposure data is greater than the exposure difference (< 20%) estimated by the Pop-PK model (11 – 21% higher CL values in ADA+ subjects).

Immunogenicity did not have apparent effect on the pharmacodynamics of asfotase alfa. No apparent trend was observed between asfotase alfa concentration and % inhibition by domain (TNSALP and FcD10).

The impact of immunogenicity on clinical efficacy cannot be evaluated adequately for both the infantile-onset HPP patients and the juvenile-onset HPP patients. For the infantile-onset subjects, the assessment of the impact of immunogenicity on overall survival was limited by the

small number of subjects who died. For the juvenile-onset HPP subjects, no assessment was performed due to the suboptimal quality of the 6MWT video recording for the gait assessment.

Immunogenicity appeared to have an impact on the rate of injection site reactions (ISR) and ectopic calcification; ADA+ subjects had a slightly higher rate of the two adverse events. Because of the small number of subjects and the short duration of the clinical trials, long term immunogenicity and safety assessments are warranted to provide further insight into the impact of immunogenicity on safety.

## 2. QUESTION BASED REVIEW

### 2.1 List the Clinical Pharmacology and the clinical studies with PK and/or PD information submitted in the BLA

A summary of asfotase alfa clinical studies to support the clinical pharmacology section of this BLA submission is presented in Table 1.

### 2.2 General Attributes of the Drug

#### 2.2.1 *What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?*

Asfotase alfa (b) (4) is a soluble fusion glycoprotein comprised of two identical polypeptide chains. Each polypeptide chain contains the catalytic domain of human TNSALP, the human IgG1 Fc domain (b) (4) and a D10 domain used for bone-targeting. The two polypeptide chains are connected by two (b) (4) disulfide bonds (b) (4). Asfotase alfa is expressed in the engineered Chinese hamster ovary (CHO) cell line (b) (4).

The drug substance for the to-be-marketed drug product is manufactured at a (b) (4) scale. During clinical development, the drug product was (b) (4) from (b) (4) to (b) (4). The (b) (4) and (b) (4) drug products were used in all the clinical studies except for Study ENB-001-08 which used only the (b) (4) product.

Per Applicant's proposal, the drug substance in the to-be-marketed drug product is to (b) (4)

**Table 1. Summary of asfotase alfa clinical studies in BLA submission**  
 (Source: Table 1 of Section 1.1.14 Clinical Pharmacology Reviewer Guide)

Study Number	Phase/ Patient Population	Study Design	Asfotase Alfa Dosing Regimen <sup>(1)</sup>	Number of Enrolled/ Completed Patients at Data Cut-off	Mean Age at Study Entry (±SD)	Gender	Participating Countries	Current Status
ENB-001-08	Phase 1 Adults	Multi-center, multinational, open-label, dose escalating, safety/efficacy, PK, PD	Single IV infusion of 3 mg/kg followed by 3 SC injections [Cohort 1 (n=3) received 1 mg/kg/week; Cohort 2 (n=3) received 2 mg/kg/week] at weekly intervals	6 enrolled, 6 completed	44.8 (12.6) years old	2 M 4 F	Canada US	Completed
ENB-002-08	Phase 2 Infants and young children (infantile onset)	Multicenter, multinational, open-label, single group assignment, safety/efficacy	Single IV infusion of 2 mg/kg followed by SC injections of 1 mg/kg 3 times per week for 23 weeks	11 enrolled, 10 completed <sup>(2)</sup>	58.8 (59.2) weeks old	4 M 7 F	Canada UAE UK USA	Completed
ENB-003-08	Phase 2 Infants and young children, Extension (infantile onset)	Multicenter, multinational, open-label, single group assignment, safety/efficacy, extension of ENB-002-08	SC injections 3 times per week at final dose received in ENB-002-08	10 enrolled, 9 receiving treatment <sup>(3)</sup>		4 M 6 F	UAE UK USA	Ongoing
ENB-006-09	Phase 2 Children and early adolescents (infantile and juvenile onset)	Multicenter, multinational, open-label, dose comparison, parallel assignment, historical control, safety/ efficacy, PK, PD	SC injections of 2 mg/kg or 3 mg/kg 3 times per week (a total of 6 mg/kg/week or 9 mg/kg/week)	13 enrolled (6 in 2 mg/kg group, 7 in 3 mg/kg group), 12 completed (6 in 2 mg/kg group, 6 in 3 mg/kg group) <sup>(4)</sup>	8.8 (2.2) years old	11 M 2 F	Canada USA	Completed
ENB-008-10	Phase 2 Children and early adolescents, Extension (infantile and juvenile onset)	Multicenter, multinational, open-label, dose comparison, parallel assignment, safety/ efficacy, PK, PD, extension of ENB-006-09	1 mg/kg 6 times per week or 2 mg/kg 3 times per week SC injections (a total of 6 mg/kg/week)	12 enrolled and receiving treatment		10 M 2 F	Canada USA	Ongoing

**Table 1. Summary of asfotase alfa clinical studies in BLA submission (Continued)**

Study Number	Phase/ Patient Population	Study Design	Asfotase Alfa Dosing Regimen <sup>(1)</sup>	Number of Enrolled/ Completed Patients at Data Cut-off	Mean Age at Study Entry (±SD)	Gender	Participating Countries	Current Status
ENB-009-10	Phase 2 Adolescents and Adults (infantile, juvenile and adult onset)	Randomized, open-label, multicenter, multinational, dose-ranging, concurrent control, safety/ efficacy, PK	0.3 mg/kg daily or 0.5 mg/kg daily or no treatment for first 24 weeks; all patients receiving 0.5 mg/kg daily starting at Week 24 for next 24 weeks; Patients then receiving 1 mg/kg 6 times a week for additional 48 weeks	19 enrolled (6 in control group, 6 in 0.5 mg/kg group, 7 in 0.3 mg/kg group); 18 receiving treatment in open label extension <sup>(5)</sup>	40.9 (20.1) years old	7 M 12 F	Canada USA	Ongoing
ENB-010-10	Phase 2 Infants and Children (infantile onset)	Open-label, multicenter, multinational, safety/efficacy, PK	SC injections of 2 mg/kg 3 times weekly or 1 mg/kg 6 times weekly (a total of 6 mg/kg/week)	28 enrolled receiving treatment	118 (113) weeks old	12 M 16 F	Canada USA DE Turkey Taiwan Japan	Ongoing

<sup>(1)</sup> For all clinical studies except ENB-001-08 and ENB-009-10, dose adjustments were allowed for lack of efficacy or safety-related concerns.

<sup>(2)</sup> Patient 002-03-01 discontinued after the IV dose and before SC study drug administration due to a moderate infusion-associated reaction.

<sup>(3)</sup> Patient 003-08-01 died on 24 September 2009, after approximately 3 months on treatment in this extension study (after approximately 9 months of asfotase alfa treatment) from SAE of septic shock which was considered unrelated to study drug.

<sup>(4)</sup> Patient 006-02-04 in the 3 mg/kg group discontinued to undergo a pre-planned scoliosis surgery.

<sup>(5)</sup> Four patients participated in ENB-001-08 study prior to the beginning of ENB-009-10 study.

Abbreviations: CSR = clinical study report; DE = Germany; F = female; FPI = first patient in; IV = intravenous; LPO = last patient out; M = male; mo. = month; NA= not applicable; PD = pharmacodynamics; PK = pharmacokinetics; SC = subcutaneous; UAE = United Arab Emirates; UK = United Kingdom; USA = United States of America; yr. = year(s)

The drug product is supplied as a sterile aqueous solution for SC administration containing asfotase alfa at a concentration of 40 mg/mL or 100 mg/mL in (b) (4) sodium phosphate, (b) (4) sodium chloride in a 2 mL glass vial. At the 40 mg/mL concentration, it is supplied as a single-use vial at (b) (4) 0.45, 0.70, and 1.0 mL volumes containing (b) (4) 18, 28, and 40 mg of asfotase alfa, respectively. At the 100 mg/mL concentration, it is supplied in a single-use vial at a 0.80 mL volume containing 80 mg of asfotase alfa.

### ***2.2.2 What are the proposed therapeutic indication and the mechanism of action?***

Asfotase alfa is indicated for (b) (4) in patients of all ages with infantile-onset and juvenile-onset HPP. The proposed indication does not include adult-onset HPP patients.

In patients with HPP, loss-of-function mutation(s) in the gene encoding TNSALP causes a deficiency in TNSALP enzymatic activity, which leads to elevated circulating levels of several TNSALP substrates including inorganic pyrophosphate (PPi) and pyridoxal-5'-phosphate (PLP). Elevated extracellular levels of PPi block hydroxyapatite crystal growth, which further inhibit bone mineralization and causes an accumulation of unmineralized bone matrix. This is manifested as rickets and bone deformation in infants and children and as osteomalacia (softening of bones) once growth plates close along with muscle weakness resulting in physical disability and impaired quality of life

Administration of asfotase alfa to patients with HPP is expected to cleave PPi, releasing inorganic phosphate for combination with calcium, thereby promoting hydroxyapatite crystal formation, bone mineralization, and restoring the normal skeletal integrity.

### ***2.2.3 What are the proposed dosing regimen and route of administration?***

The proposed dosing regimen is 6 mg/kg per week by SC injection, to be administered either as 2 mg/kg 3x/week or 1 mg/kg 6x/week as SC injections.

### ***2.2.4 What drugs (substance, products) are approved for the same indication in the US?***

Currently, there are no approved drug products for the same indication in the US.

## **2.3 General Clinical Pharmacology**

### ***2.3.1 What are the design features of the clinical pharmacology studies and the clinical studies used to support dosing or claims?***

The Applicant seeks marketing approval of asfotase alfa based on efficacy/PD data from 5 interventional clinical trials and 2 extension trials (Studies ENB-001-08, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, ENB-009-10, and ENB-010-10) as well as historical data from 2 natural history studies (Studies ENB-011-10 and ALX-HPP-502). Table 2 contains a brief summary of the study design for these studies. All of the interventional clinical trials were open-label studies.

**Table 2. Interventional clinical studies with asfotase alfa in HPP patients**

(Source: Tables 5 and 7 of Clinical Pharmacology Reviewer Guide)

Study	ENB-001-08	ENB-002-08/ ENB-003-08	ENB-006-09/ ENB-008-10	ENB-009-10	ENB-010-10
Study Design	MC, OL, dose-escalating FIH study	MC, OL study and extension	MC and OL study and extension with HC	Randomized, MC, OL study	MC, OL study
Region	US, CAN	Global	USA, CAN	USA, CAN	Global
Age at Inclusion	adult	< 3 years	5-12 years	>12 years	≤5 years
Number of Patients	6	11	13	19 <sup>a</sup>	28
Perinatal/Infantile <sup>b</sup>	1	11	5	4	28
Juvenile <sup>b</sup>	3	0	8	12	0
Adult <sup>b</sup>	0	0	0	2	0
Unknown <sup>c</sup>	2	0	0	1	0
Follow-up Time	NA	≥ 36 months	≥ 36 months	≥ 18 months	≥ 12 months
Dosing Route	IV and SC	IV and SC	SC	SC	SC
Dosing Regimen	<i>Week 1:</i> 3 mg/kg IV,  <i>Weeks 2-4:</i> Cohort 1: 1 mg/kg SC weekly  Cohort 2: 2 mg/kg SC weekly	<i>Week 1:</i> 2 mg/kg IV,  <i>Weeks 2-24:</i> 1 mg/kg SC 3x/week, with adjustment as needed (to 2 mg/kg 3x/week at month 1 and to 3 mg/kg 3x/week at month 4)	<i>Weeks 1-24:</i> 2 mg/kg 3x/week or 3 mg/kg 3x/week, with adjustment as needed;  Later amended to 2 mg/kg 3x/week or 1 mg/kg 6x/week, with dose adjustments as needed	<i>Weeks 1-24:</i> 0.3 mg/kg daily or 0.5 mg/kg daily or no treatment;  <i>Weeks 25-48:</i> 0.5 mg/kg daily  48 more weeks: 1 mg/kg 6x/week	2 mg/kg 3x/week or 1 mg/kg 6x/week with adjustment as needed

**Table 2. Clinical studies with asfotase alfa in HPP patients (Continued)**

Study	ENB-001-08	ENB-002-08/ ENB-003-08	ENB-006-09/ ENB-008-10	ENB-009-10	ENB-010-10
Primary endpoint by study	PK	RGI-C	RGI-C	PLP, PPi	RGI-C
Selected secondary endpoint that were used for PK/PD analysis by Study	PLP, PPi	OS, VFS, PK, PLP, PPi, RSS, BOT-2, Growth	PK, PLP, PPi, RSS, 6MWT, BOT-2, Growth	PK, PLP, PPi, RSS, 6MWT, BOT-2	OS, VFS, PK, PLP, PPi, RSS, BOT-2, Growth
Primary efficacy endpoint for submission	NA	OS and VFS compared to NH study ENB-011-10	RGI-C compared to ENB-011-10; MPOMA-G compared to NH study ALX-HPP-502s	NA	OS and VFS compared to NH study ENB-011-10

<sup>a</sup>Four patients were from ENB-001-08 study

<sup>b</sup>Age of disease onset

<sup>c</sup>These patients were adults at the time of enrollment, but the age of disease onset was unknown.

MC = multi-center; OL = open label; NH = natural history; CAN = Canada; HPP = hypophosphatasia; IV = intravenous; SC = subcutaneous; USA = United States of America; FIH = first in Human; HC = historic control; TIW = 3 times weekly; NA: not applicable; PPi = Inorganic pyrophosphate; PLP = Pyridoxal-5'-phosphate; RSS = Rickets severity scale; RGI-C = Radiographic Global Impression of Change; BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; 6MWT = 6-minute walk test; PDMS-2 = Peabody Developmental Motor Scales, Second Edition; BSID-III = Bayley Scales of Infant and Toddler Development, Third Edition; BPI-SF = Brief Pain Inventory-Short Form; HHD = Hand-held dynamometry; DEXA = Dual-energy X-ray absorptiometry; CHAQ = Child Health Assessment Questionnaire; FVC = Forced vital capacity; PFT = Pulmonary function testing; LEFS = Lower Extremity Functional Scale; POSNA PODCI = Pediatric Orthopedic Society of North America's Pediatric Outcomes Data Collection Instrument; MPOMA-G = Modified Tinetti performance oriented mobility assessment - gait subtest; OS = Overall survival; VFS = Ventilator free survival.

### 2.3.1.1 Patient Population

For each clinical study, patients were enrolled based on their age at inclusion. However, patients are classified into different HPP subtypes based on the age at onset of first signs and/or symptoms, i.e., perinatal, infantile, juvenile, and adult forms (Table 3).

**Table 3. Age at onset of first signs and/or symptoms for the different forms of HPP**

(Source: Table 4 of Section 1.1.14 Clinical Pharmacology Reviewer Guide)

Disease Form	Age at Onset of First Signs/Symptoms
Perinatal	In utero
Infantile	< 6 months of age
Juvenile	≥ 6 months to ≤ 18 year of age
Adult	> 18 years of age

ENB-001-08 enrolled only adults (n = 6), and ENB-009-10 enrolled both adolescent (n = 6) and adult subjects (n = 13; 4 of them were also enrolled in Study ENB-001-08). ENB-006-09/ ENB-008-10 enrolled pediatric patients 5 – 12 years of age. These studies mainly consist of a mixture of infantile-onset and juvenile-onset HPP patients. Two adult-onset HPP patients were enrolled in Study ENB-009-10. One and two subjects with unknown disease subtype participated in Studies ENB-009-10 and ENB-001-08, respectively (Table 2). ENB-002-08/ ENB-003-08 and ENB-010-10 enrolled only infantile-onset HPP patients ≤ 5 years old.

Data from a total of 73 subjects were initially submitted to this BLA; 15 of them were adults and 58 were pediatric patients. Upon Agency's information request (IR) dated March 10, 2015, the BLA was updated to include 102 patients with HPP.

### 2.3.1.2 Dosing Regimen

Among the five interventional trials, only ENB-010-10 evaluated the proposed dosing regimen of 2 mg/kg SC 3x/week or 1 mg/kg SC 6x/week starting at the beginning of the study. The remaining studies initially used regimens other than the proposed dosing regimen of 6 mg/kg SC every week. For patients in ENB-006-09/ENB-008-10 and ENB-009-10, the initial dosing regimens were later adjusted to the proposed dosing regimens of 6 mg/kg SC every week (Table 2).

### 2.3.1.3 Response Endpoint

Multiple endpoints were assessed in the clinical studies (shown in Table 2), and some of them were used by the Applicant to provide the PK/PD rationale for the selection of the proposed dosing regimen (shown in Table 4).

**Table 4. Summary of endpoints evaluated by population PK-PD/Efficacy modeling**

	<b>Response Endpoints</b>	<b>Phenotype</b>	<b>PK/PD Findings as Rationale to Support Proposed Dose Regimen</b>
PD (Biochemical)	PPi	Infantile-, juvenile-, adult-onset	Show effect on plasma PPi and PLP plateauing out
	PLP	Infantile-, juvenile-, adult-onset	
PD (Radiologic)	RGI-C	Infantile-, juvenile-onset	Show near maximal change from baseline
	RSS (Knee and Wrist)	Infantile-, juvenile-onset	
PD (Functional)	BOT-2	Juvenile-onset	Indicate significant improvement in ambulation
	6MWT	Juvenile-onset	
Efficacy	MPOMA-G	Juvenile-onset	Improve survival
	Overall survival	Infantile-onset	

PD, pharmacodynamics; PPi, inorganic pyrophosphate; PLP, pyridoxal-5'-phosphate; RGI-C, Radiographic Global Impression of Change; RSS, rickets severity scale; BOT-2, Bruininks-Oserestsky Test of Motor Proficiency, Second Edition; 6 MWT, 6 minute walk-test; MPOMA-G, modified Tinetti performance oriented mobility assessment-gait score; (b) (4)

\* see Bioanalytical Assays section below

### 2.3.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology studies?

The rationales for selecting the response endpoints are discussed in sub-sections below organized by the type of responses, i.e., biochemical, radiological, functional, and efficacy endpoints.

#### 2.3.2.1 Biochemical Endpoints

PPi and PLP are biochemical PD markers because they are substrates of alkaline phosphatase and are known to be elevated in HPP patients. Administration of asfotase alfa is expected to hydrolyze these substrates, thereby normalize PPi and PLP levels and improve bone mineralization.

The PPi assay utilizes a (b) (4) method based on (b) (4). The Applicant initially used a (b) (4). See Sections 2.9.3 and 2.9.4 for more details.

PLP was initially (b) (4). See Section 2.9.5 for further details.

#### 2.3.2.2 Radiological Endpoints

Radiological endpoints were selected because failure to mineralize bone matrix manifests in infants and children with HPP as rickets, including bowed legs or knock-knees, enlargement of the wrists, knees, and ankles from flared metaphyses, and beading of the costochondral junctions.

To measure the impact of asfotase alfa on these skeletal structural manifestations of HPP, skeletal X-rays were obtained prior to initiation of asfotase alfa treatment and post Baseline in all pediatric patients <18 years of age with open growth plates, and were assessed by RGI-C and RSS scores.

RGI-C scale provides an overall evaluation of change in characteristic skeletal manifestations of HPP. It is a 7-point scale ranging from a value of -3 (indicative of severe worsening of HPP-associated rickets) to a value of +3 (indicative of complete or near complete healing of HPP-associated rickets). Three independent radiologists assessed and compared skeletal X-rays of the chest, bilateral wrists, and bilateral knees (depending on the study) obtained prior to and following initiation of treatment and assigned an overall change from Baseline RGI-C score (and in some cases regional change scores for the hands/wrists and knees).

RSS evaluates the severity of rickets. It has a maximum total score of 10 points, with a maximum score of 4 points for the wrists and 6 points for the knees. A score of 10 represents severe rickets, while a score of 0 represents an absence of cupping and fraying. A single independent expert rater who was blinded to assessment time point and all other patient information read all patient X-rays and completed the RSS scoring. Effect of asfotase alfa treatment is to be evaluated based on the comparison the RSS scores before and after asfotase alfa treatment.

#### 2.3.2.3 Functional Endpoints

Because gross motor deficits along with ambulatory compromise have been observed in older children and adults with HPP, BOT-2 and 6MWT have been administered to assess changes in these clinical features.

BOT-2 score identifies motor skills deficits in patients with mild to moderate motor control problems. It employs engaging, goal-directed activities to measure a wide array of skills in children and young adults from 4 to 21 years of age. In the asfotase alfa treatment studies, BOT-2 was administered by trained and qualified study personnel, focusing on those subtests that evaluate functions most affected by HPP. For each subtest, total, scaled, and age-equivalent scores were recorded. BOT-2 age-equivalent scores indicate the average age at which healthy children typically achieve the raw score obtained by the patient on a given scale, while scaled scores reflect the patient's performance relative to healthy, same-aged peers. The normal mean (SD) for BOT-2 scaled scores is 15 (5).

The 6MWT evaluates the effects of asfotase alfa on mobility, specifically ambulation. In asfotase alfa studies enrolling patients  $\geq 5$  years of age (i.e., Studies ENB-006-09/ENB-008-10 and ENB-009-10), the 6MWT was administered by trained and qualified study personnel in accordance with American Thoracic Society (ATS) guidelines (ATS 2002) prior to study drug administration and any bone biopsy procedure (when applicable). The primary measurement was the distance walked in meters. The percent predicted 6MWT values were calculated by expressing observed 6MWT distance for each patient as a percentage of those observed in sex-, age-, and height-matched healthy individuals from a normative sample.

#### 2.3.2.4 Efficacy Endpoints

Overall survival and ventilation free survival were the efficacy endpoints for the infantile-onset subgroup. For the juvenile-onset subgroup, gait performance was the efficacy endpoint and was assessed by a modified Tinetti Performance-Oriented Mobility Assessment (POMA) scale.

POMA is a tool consisting of two subtests: POMA-G (Gait) and the POMA-B (Balance). A modified version of the POMA-G (MPOMA-G) was used to assess clinical efficacy of asfotase alfa by comparing the gait performance in patients with juvenile-onset HPP in Studies ENB-006-09/ENB-008-10 to historical control patients from Study ALX-HPP-502s. The POMA-G measures step length and height, step symmetry and continuity, foot clearance, trunk sway, and stance, all of which may be abnormal in HPP patients secondary to skeletal instability, poor alignment, and proximal and lower extremity weakness characteristic of the disease. Zero is the lowest score per item, and 1, or in some cases 2, is the highest score per item, with a maximum total score of 12.

Video recordings of the 6MWT obtained as part of Studies ENB-006-09/ENB-008-10 were the basis for evaluating gaits in treated patients. The MPOMA-G and POMA-G scores were rated by three qualified and trained physical therapists who did not participate in caring for enrolled patients and were masked to patient identifiers and the dates of video recording. Video footage taken as part of routine clinical care at the St. Louis Shriners Hospital site were used as the basis for evaluating gait in historical control patients in Study ALX-HPP-502s. For each item the median of the 3 rater's scores was determined and the medians were summed to determine total score.

### **Reviewer's Comments**

*The review team identified certain limitations of the 6MWT video recordings for the gait assessment in juvenile-onset patients and considered it helpful to use growth as an additional clinical endpoint for evaluation of asfotase alfa clinical efficacy. For this reason, the E-R relationship for growth rate is included in this review. The E-R relationship of MPOMA-G score can be found in the Pharmacometrics Review.*

#### **2.3.3 Are the active moieties in plasma appropriately identified and measured to assess pharmacokinetic (PK) parameters and exposure response relationships?**

Asfotase alfa is the active moiety and its concentrations were measured as enzyme activity in serum. Measurement of serum asfotase alfa concentrations in activity was appropriate.

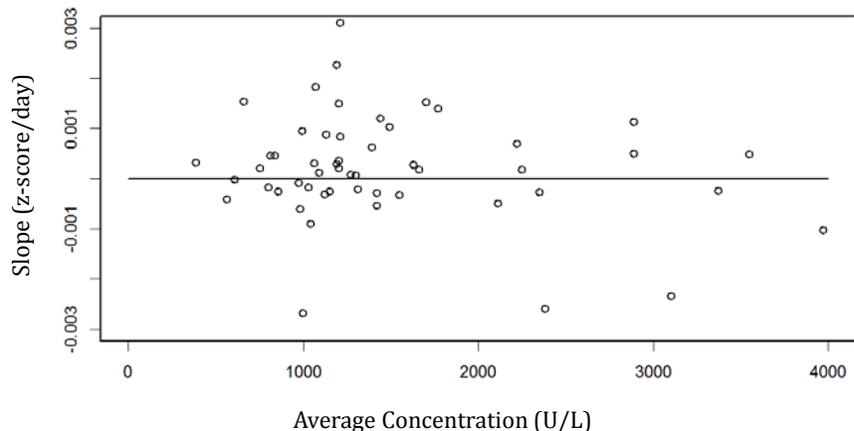
### **2.4 Exposure-Response**

#### **2.4.1 What are the characteristics of the E-R relationships for effectiveness?**

Using a population PK-PD modeling approach, the Applicant performed several E-R relationship evaluations for effectiveness in patients with HPP. The endpoints assessed and the respective patient population data evaluated are presented in Table 4.



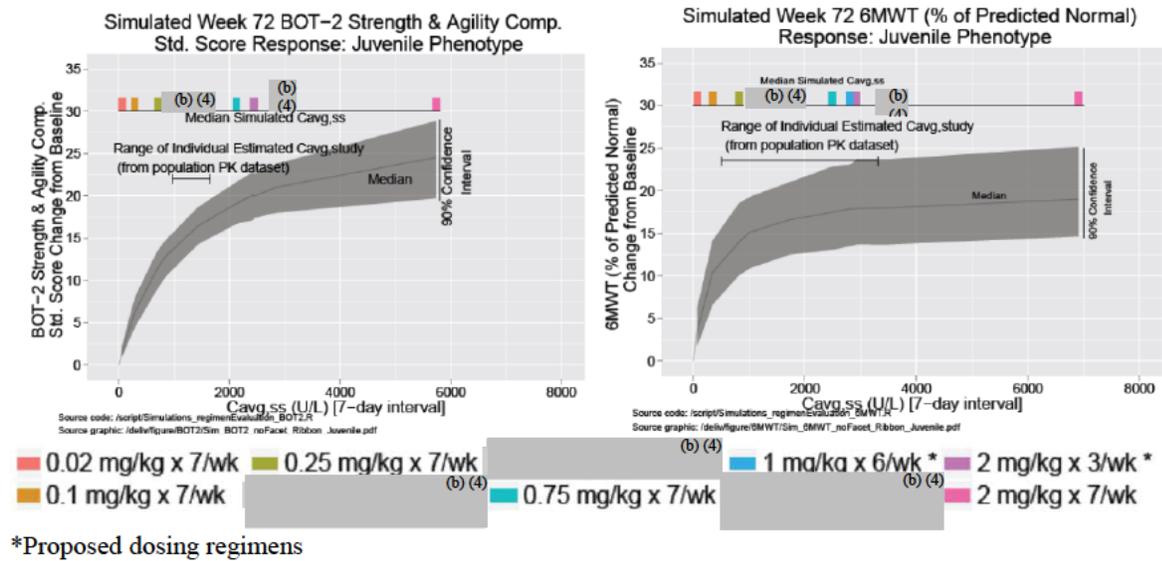
**Figure 2. E-R analysis for growth rate. The open circles depict the slope of the z-score over the duration of treatment for each individual and the solid line is a reference line at zero.**



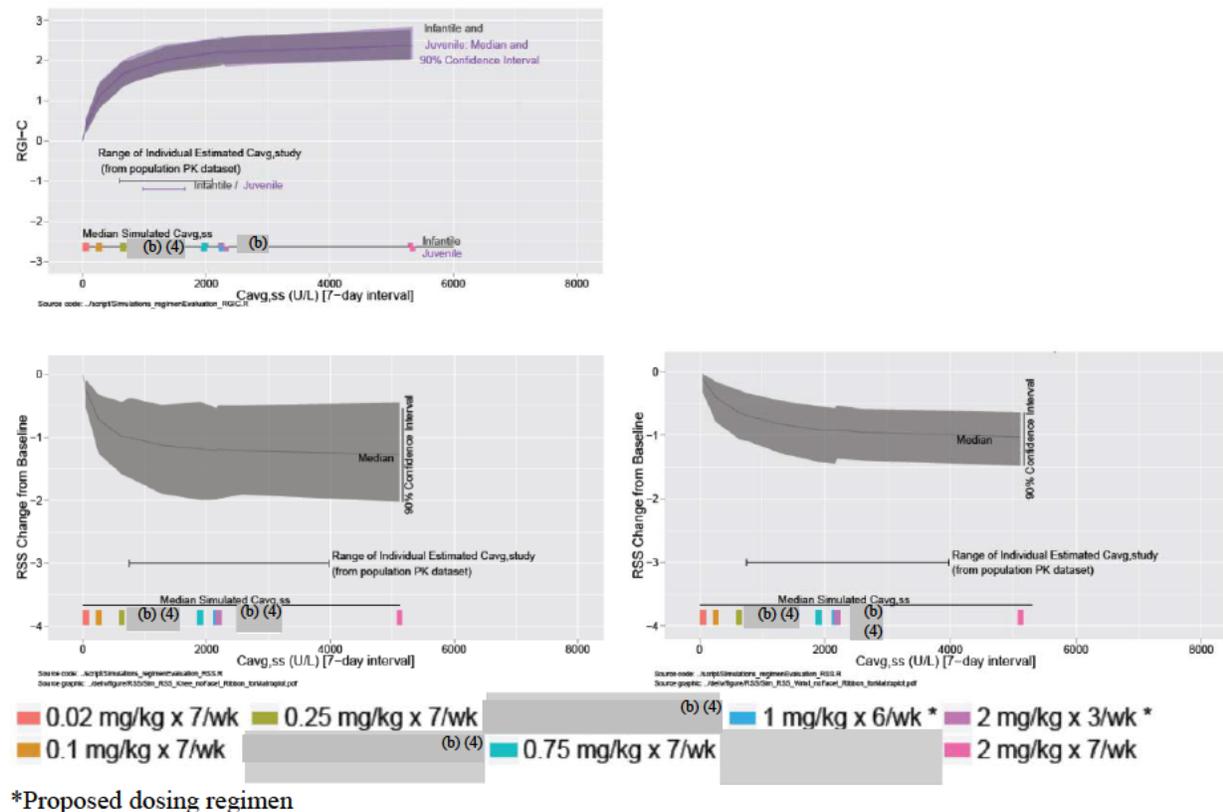
#### 2.4.1.2 Additional Endpoints

Using the indirect response model(s), the Applicant conducted population PK-PD modeling for six additional endpoints (BOT-2, 6MWT, RGI-C, RSS, PPI, and PLP) to support the efficacy of asfotase alfa in HPP patients. The E-R curve for six endpoints resembles an  $E_{max}$  curve; greater improvement of response was observed in the initial part of the E-R curve at lower concentrations, with response plateauing at higher asfotase alfa concentrations of 1500 – 2000 U/L. Of note, the initial part of the E-R curves for RGI-C and RSS scores was not as steep as the initial slope of the E-R curves for the other four endpoints. Figure 3 presents the E-R relationship for BOT-2 and 6MWT in juvenile-onset patients at Week 72. The E-R curves for RGI-C, RSS knee, and RSS wrist at Week 72 as well as those for PPI at Week 7 and PLP at Week 24 in infantile- and juvenile-onset patients are depicted by Figure 4 and Figure 5, respectively.

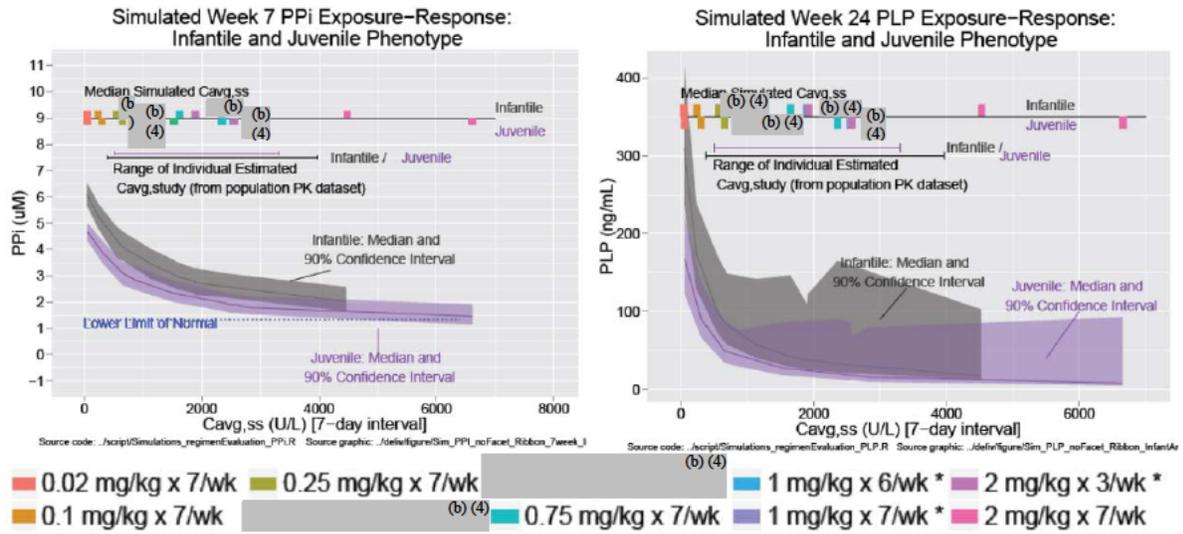
**Figure 3. Simulated E-R curves for BOT-2 score and 6MWT**  
 (Source: Figure 4 of Population PKPD Report)



**Figure 4. Simulated E-R curves for RGI-C, RSS knee, and RSS wrist**  
 (Source: Figure 3 of Population PKPD Report)



**Figure 5. Simulated E-R curves for PPI and PLP levels**  
(Source: Figure 2 of Population PKPD Report)



\*Proposed dosing regimens

#### 2.4.2 What are the characteristics of the E-R relationships for safety?

The E-R relationships for three AEs (ectopic calcification, infusion/injection associated reactions [IAR], and ISR) were assessed by quartile of asfotase alfa exposures; a total of 897 events occurred across the three safety endpoints. Table 5 revealed no apparent associations between the incidence of the specific AEs and  $C_{avg}$ .

**Table 5. Rate of AE incidence by quartile of asfotase alfa exposures ( $C_{avg}$ ) in HPP patients**  
(Source: Table 17 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Adverse Event		Quartile 1	Quartile 2	Quartile 3	Quartile 4
	$C_{avg, study}^a$ (ng/mL)	383 - 1002	1002 - 1240	1240 - 1666	1666 - 3975
	Total subjects in quartile	17	17	17	17
Ectopic Calcification	Mean rate (95 % CI) <sup>b</sup>	0.14 (0.093 - 0.24)	0.017 (0.011 - 0.029)	0.15 (0.1 - 0.26)	0.16 (0.1 - 0.27)
Injection/Infusion Associated Reactions	Mean rate (95 % CI) <sup>b</sup>	0.72 (0.47 - 1.2)	0.18 (0.12 - 0.32)	0.12 (0.078 - 0.2)	0.76 (0.5 - 1.3)
Injection Site Reactions	Mean rate (95 % CI) <sup>b</sup>	8.6 (5.6 - 15)	3.2 (2.1 - 5.4)	7.2 (4.7 - 12)	3.2 (2.1 - 5.4)

<sup>a</sup>  $C_{avg}$ , over the entire study; <sup>b</sup> Mean rate of incidence, events/year (95 % CI).

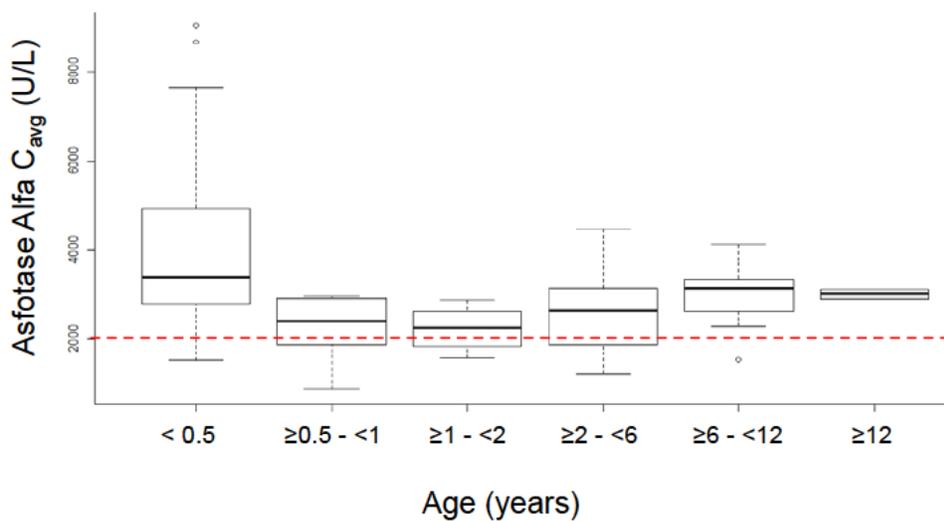
#### 2.4.3 Does this drug prolong QT/QTc interval?

No formal QT/QTc studies were performed for asfotase alfa. As the drug is a therapeutic protein circulating as a (b)(4) with a molecular weight of (b)(4) the risk for prolongation of QT/QTc interval is minimal.

#### 2.4.4 Is the dose and dosing regimen selected consistent with the known E-R relationship?

Yes, the proposed dosing regimen of 6 mg/week administered as either 2 mg/kg SC 3x/week or 1 mg/kg SC 6x/week are consistent with the E-R relationships for effectiveness of asfotase alfa based on multiple endpoints. As demonstrated in Figure 6, the proposed regimen results in median steady state concentrations of approximately  $\geq 2000$  U/L in different patient age groups, which appear to fall in the plateau of the exposure-response curves for BOT-2 score (Figure 3), 6MWT (Figure 3), RGC-I (Figure 4), RSS (Figure 4), PPI (Figure 5), and PLP (Figure 5).

**Figure 6. Average Asfotase Alfa Concentration versus Age. Asfotase Alfa  $C_{avg}$  was calculated from the individual post hoc Bayesian estimates of clearance for each individual, and the average dose received over the duration of treatment.**



#### 2.5 What are the PK characteristics of the drug?

##### 2.5.1 Data from which study were used to determine the PK characteristics of the drug?

As of data cut-off date of November 22, 2013 for the initial BLA submission, asfotase alfa concentration data were available from 73 HPP subjects enrolled in all of the clinical trials and were used to determine the PK characteristics of asfotase alfa using non-compartmental analysis (NCA) and Pop-PK analysis.

Table 6 summarizes the available PK data as well as the associated dosing regimens for each study.

**Table 6. Types of PK data available for each clinical trial**

	ENB-001-08	ENB-002-08/ ENB-003-08	ENB-006-09/ ENB-008-10	ENB-009-10	ENB-010-10
Patient group	Adults	Infants and young children	Children and early adolescents	Adolescents and adults	Infants and children ≤ 5
Dosing regimen 1	3 mg/mg IV infusion (Week 1)	2 mg/kg IV infusion (Week 1)	2 or 3 mg/kg SC 3x/week x 24 weeks	0.3 or 0.5 mg/kg SC QD or no treatment x 24 weeks	1 mg/kg 6x/week or 2 mg/kg 3x/week (max dose = 40 mg)
PK data 1	Pre-dose, during, and at end of infusion, then 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72 hours	Pre-dose and 1, 4, 12, 24, 48, and 96 hours	<ul style="list-style-type: none"> <li>• Baseline and Week 6: Pre-dose and at 6, 12, 24, 32, and 48 hours</li> <li>• Weeks 12, 18, and 24: pre-dose</li> </ul>	<ul style="list-style-type: none"> <li>• Baseline and Weeks 3, 12, and 24: pre-dose and 2, 4, 8, 12, 16, and 24 hours</li> <li>• Week 6: pre-dose</li> </ul>	<ul style="list-style-type: none"> <li>• Baseline and Week 6: pre-dose, 6, 12, 24, 32, and 48 hours</li> <li>• Months 3, 6, 9, 12, 15, 18, 24, 30, 32, 36, 42, 48: pre-dose</li> </ul>
Dosing regimen 2	1 or 2 mg/kg SC QW x 3 (Weeks 2–4)	1 mg/kg SC 3x/week x 3 weeks	1 mg/kg SC 6x/week or 2 mg/kg SC 3x/week	0.5 mg/kg SC QD x 24 weeks	NA
PK data 2	<ul style="list-style-type: none"> <li>• 1<sup>st</sup> SC dose: pre-dose, 6, 12, 24, 48, 72 hours</li> <li>• 2<sup>nd</sup> SC dose: pre-dose and 24 hours</li> <li>• 3<sup>rd</sup> SC dose: pre-dose, 6, 12, 24, 48, 72, 168 hours</li> </ul>	SC doses: pre-dose and 6, 12, 18, 24, 36, and 48 hours (n = 7)	Baseline, Months 3, 6, and 9, and every 6 months thereafter: pre-dose	NA	NA
Dosing regimen 3	NA	2 mg/kg 3x/week x 3 months	NA	1 mg/kg 6x/week x 48 weeks	NA
PK data 3	NA	2 <sup>nd</sup> and 5 <sup>th</sup> SC doses: pre-dose	NA	NA	NA
Dosing regimen 4	NA	Dose adjustments up to 3 mg/kg 3x/week	NA	NA	NA
PK data 4	NA	<ul style="list-style-type: none"> <li>• 6<sup>th</sup> SC dose or subsequent dose: pre-dose and 6, 12, 18, 24, 36, and 48 hours, and 72 hours if days between doses</li> <li>• Weeks 4, 12, 16, and 24: pre-dose</li> </ul>	NA	NA	NA

### 2.5.2 What's the absolute bioavailability of asfotase alfa when administered subcutaneously?

The estimated absolute bioavailability after SC administration of asfotase alfa ranged from 45.8 to 98.4% in six adult HPP patients enrolled in Study ENB-001-08. All six adult HPP patients were Caucasians (4 females, 2 males) with a mean  $\pm$  SD age of  $44.8 \pm 12.6$  years old and a mean weight of  $70.6 \pm 16.5$  kg. All subjects (2 cohorts of 3 subjects each) received an IV dose of asfotase alfa at 3 mg/kg in the first week followed by three SC doses of either 1 mg/kg (cohort 1) or 2 mg/kg (cohort 2) asfotase alfa administered at weekly interval (QW) from Week 2 to Week 4. The asfotase alfa concentration-time profiles are shown in (Figure 7).

**Figure 7. Mean serum asfotase alfa activity (U/L) vs. time profiles in ENB-001-08 study**  
(Source: Figure 1 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

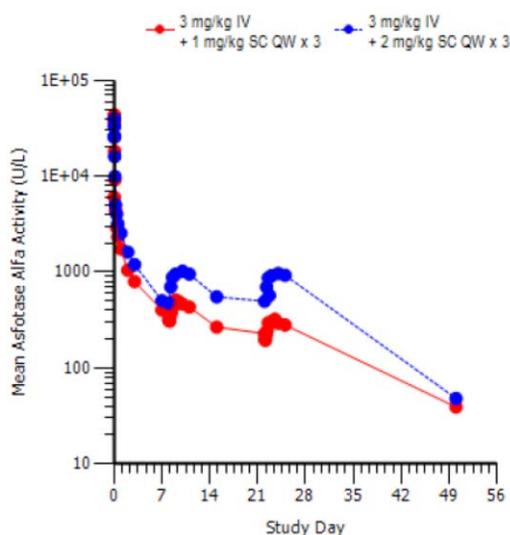


Table 7 summarizes the PK parameter values following IV and SC administration of asfotase alfa determined using NCA method. The absolute bioavailability after the first and third SC administration ranged from 45.8 to 98.4%. The mean apparent terminal elimination half-life ( $t_{1/2}$ ) of asfotase alfa ranged from 112 to 135 hours after SC administration. This  $t_{1/2}$  after SC administration was longer than that following IV administration, suggesting absorption rate limited elimination. Because of the small sample size, no formal statistical analysis was conducted for assessing linearity of the non-compartmental PK parameters of asfotase alfa.

**Table 7. Asfotase alfa PK parameters in patients with HPP who participated in Study ENB-001-08**  
(Source: Table 5 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

PK Parameters <sup>a</sup>	Cohort 1 (N=3)			Cohort 2 (N=3)		
	IV (3 mg/kg)	SC (1 mg/kg) QW x 3		IV (3 mg/kg)	SC (2 mg/kg) QW x 3	
	Week 1	Week 2	Week 4	Week 1	Week 2	Week 4
C <sub>max</sub> (U/L)	42694 (8443)	514 (119)	340 (206)	46890 (6635)	1081 (65.2)	1020 (326)
t <sub>max</sub> (h)	1.25	24.2	35.9	1.50	48.0	48.1
AUC <sub>∞</sub> (h*U/L)	265798 (95160)	NR	NR	368978 (16615)	NR	NR
AUC <sub>t</sub> (h*U/L)	232571 (88022)	66034 (19241)	40444 <sup>b</sup>	327737 (15937)	138595 (6958)	136109 (41875)
Abs. Bio. (%)	NA	Range 62.9% – 98.4%	61.6% <sup>b</sup>	NA	Range 54.2% – 71.3%	Range 45.8% – 90.3%
Half-life (h)	72.8 (8.75)	112 (1.54) <sup>c</sup>	120 <sup>b</sup>	58.8 (7.88)	-- <sup>d</sup>	135 (27.8)

<sup>a</sup>PK parameters were taken from ENB-001-08 CSR. Data are presented in mean (SD) values except that median values were reported for t<sub>max</sub>

<sup>b</sup>n=1

<sup>c</sup>n=2

<sup>d</sup>Parameter could not be estimated.

### 2.5.3 What are the single dose and multiple dose PK parameters of asfotase alfa following SC administration in patients with HPP?

In the Alexion's response to FDA information request (IR) dated January 26, 2015, the Applicant provided datasets for PK parameters resulted from <sup>(b) (4)</sup> method following single and multiple administration of asfotase alfa in patients with HPP. Most of the data can be separated into three distinct sets according to the following characteristics (Table 8):

**Table 8. Summary of data characteristics in PK parameter datasets provided in Applicant's response to FDA IR dated January 26, 2015**

	Data Set 1	Data Set 2		Data Set 3
Study	ENB-002-08	ENB-006-09/ ENB-008-10	ENB-010-10	ENB-009-10
Formulation Strength (mg/mL)	40	100	40	100
Batch size (L)	<sup>(b) (4)</sup>			
Age of Patients (years)	< 3	5 - 12	≤ 5	> 12
Dosing Frequency <sup>a</sup>	Week 1: IV x 1 Weeks 2 – 24: SC 3x/week	Weeks 1 - 24: SC 3x/week	SC 3x/week or 6x/week	SC daily
PK Assessment Week	1, 2, 3	1, 6		1, 3, 12, 24
PK Sampling Duration	Week 1: 96 hours Week 2 - 3: 48 hours	48 hours	48 hours or 24 hours <sup>b</sup>	24 hours
PK Assay Method	<sup>(b) (4)</sup>			

<sup>a</sup>See Table 2 for more information, <sup>b</sup>only 48-hour data were used for analysis

Only PK parameters from the <sup>(b) (4)</sup> product are included in this section of the review for the purpose of comparison across age groups because PK data for <sup>(b) (4)</sup> product was available in only a few subjects. In addition, only 48-hour AUC data from ENB-006-09/ENB-008-10 and ENB-010-10 are used for comparison. Data from Study ENB-002-08 are not included because

concentration data were obtained from two PK assay methods. See Section 2.9.2 for more information.

For the purpose of comparison, normalized  $AUC_t$  value over the dosing interval ( $AUC_{t,nor}$ ) was calculated by dividing the  $AUC_t$  value by the total dose in activity units. The total dose in activity units was obtained by multiplying the administered dose amount in mg/kg by the specific activity of the batch of the drug used (U/mg). Because the half-life of asfotase alfa was approximately 5 days (see Section 2.5.2.1 above), the actual  $AUC_t$  values over the dosing interval obtained at Week 3 or after are considered to be at steady state and are equivalent to the  $AUC_{\infty}$  of a single dose.

The final PK parameter dataset used for analysis included 38 HPP patients enrolled in Studies ENB-006-09, ENB-010-10, and ENB-009-10. These studies used a daily SC dosing regimen or a three-times-per-week SC dosing regimen. Twenty two of the 38 patients were infantile-onset patients, 14 were juvenile-onset patients, one was an adult-onset patient, and the remaining patient had unknown disease onset status. Seventeen of the patients were males, 21 were females. All except one were Caucasians. The mean age of the patients was 18.1, with a range of 1 month to 66 years old. The mean weight at baseline was  $34.1 \pm 27.9$  kg.

#### 2.5.3.1 PK Parameters Following SC Administration of the First Dose in HPP Patients

Single dose PK data were available after the first dose at Week 1 for 2 to 3 mg/kg SC dose of asfotase alfa in Studies ENB-006-09/ENB-008-10 and ENB-010-10 and for 0.3 to 0.5 mg/kg SC dose in Study ENB-009-10 (Table 9).

The mean  $t_{max}$  ranged from 16 to 37 hours, and asfotase alfa concentrations were measurable at the end of dosing interval in all subjects. Of note, PK samples were collected up to 32 hours during Week 1 in Study ENB-010-10. Based on all data combined, the  $C_{max}$  and  $AUC_t$  over a dosing interval appeared to increase dose proportionally over the dose range of 0.3 mg/kg to 3 mg/kg. Additionally, the  $AUC_{t,nor}$  over a dosing interval were similar which is suggestive of a similar PK across the age range of the studied patients.

#### 2.5.3.2 PK Parameters Following SC Multiple Dosing Administration in HPP Patients

Multiple dose PK data were available at Week 6 after 2 to 3 mg/kg SC 3x/week dosing in Studies ENB-006-09/ENB-008-10 and ENB-010-10 and at Weeks 3, 12, and 24 after 0.3 to 0.5 mg/kg SC daily dosing in Study ENB-009-10.

After multiple dosing for 3 to 6 weeks, the  $C_{max}$  and  $AUC_t$  over a dosing interval were higher than those observed at Week 1 (Table 10). The accumulation ratios were 5 - 6 for daily dosing at 0.3 to 0.5 mg/kg dose and were 2.5 - 4 for 3 times weekly dosing at 2 - 3 mg/kg. Similar mean  $AUC_{t,nor}$  was observed among Week 3, 12, and 24, suggesting that steady state exposure was achieved as early as at Week 3 (refer to Section 2.5.7).

**Table 9. Summary of PK parameters following single dosing of asfotase alfa in HPP patients at Week 1 by dose by study**  
(Source data submitted in Alexion's response to FDA IR dated January 26, 2015)

Dose	0.3 mg/kg	0.5 mg/kg	2 mg/kg	2 mg/kg	3 mg/kg
Study ID, Study Week	ENB-009-10, 1	ENB-009-10, 1	ENB-006-09, 1	ENB-010-10, 1	ENB-006-09, 1
N	1	2	6	12	7
(b) (4)					
Age (years)	15	34.0 ± 26.9 34.0 (15.0 – 53.0)	7.8 ± 2.3 7.5 (5.0 – 12.0)	3.3 ± 2.2 3.6 (0.1 – 5.9)	8.9 ± 2.3 10.0 (6.0 – 12.0)
Weight at baseline (kg)	52	65.8 ± 7.8 65.8 (60.2 – 71.3)	21.2 ± 7.9 20.6 (11.4 – 35.4)	11.3 ± 5.2 12.4 (3.2 – 17.1)	30.6 ± 16.4 27.7 (17.4 – 62.6)
t <sub>last</sub> (h)	23.9	24.0 ± 0.014 24.0 (24.0)	47.9 ± 0.24 48.0 (47.5 – 48.1)	31.7 ± 0.79 <sup>c</sup> 32.0 (30.0 – 32.2)	48.1 ± 0.14 48.0 (47.9 – 48.3)
t <sub>max</sub> (h)	23.9	16.0 ± 0.014 16.0 (16.0)	37.3 ± 8.1 32.1 (31.8 – 48.0)	26.5 ± 7.6 30.9 (12.0 – 32.2)	34.4 ± 6.0 32.1 (31.9 – 48.0)
C <sub>max</sub> (U/L)	219	391 ± 66.6 391 (344 - 438)	566 ± 120 555 (390 - 761)	975 ± 775 730 (537 - 3371)	1257 ± 439 1205 (797 - 2323)
AUC <sub>t</sub> (h*U/L)	3530	6530 ± 1069 6530 (5773 - 7285)	19714 ± 5723 18437 (13283 - 30445)	24406 ± 20052 19143 (13045 - 86168)	45247 ± 15119 46629 (29167 - 72838)
C <sub>max,nor</sub> (U/L)/(U/kg)	0.889	0.963 ± 0.168 0.98 (0.87 – 1.1)	0.31 ± 0.07 0.30 (0.21 – 0.42)	0.69 ± 0.65 0.48(0.34 – 2.7)	0.45 ± 0.15 0.43 (0.29 – 0.75)
AUC <sub>t,nor</sub> (h*U/L)/(U/kg)	14.3	16.4 ± 2.69 16.4 (14.5 – 18.3)	10.7 ± 3.2 10.0 (7.1 – 16.7)	17.3 ± 16.8 12.3 (7.6 – 69.0)	16.2 ± 5.3 16.8 (10.5 – 25.8)

<sup>a</sup>Mean ± S.D., <sup>b</sup>Median (Range), <sup>c</sup>The last sample was collected at 32 hours for the data in Week 1

**Table 10. Summary of PK parameters following multiple dosing of asfotase alfa at Week 3 or Week 6 by dose by study**  
(Source data submitted in Alexion's response to FDA IR dated January 26, 2015)

Dose	0.3 mg/kg	0.5 mg/kg	2 mg/kg	2 mg/kg	3 mg/kg
Study ID, Study Week	ENB-009-10, 3	ENB-009-10, 3	ENB-006-09, 6	ENB-010-10, 6	ENB-006-09, 6
N	6	6	6	15	5
(b) (4)					
Age (years)	43.7 ± 23.4 51.0 (14.0 – 66.0)	48.5 ± 16.5 55.0 (15.0 – 57.0)	7.8 ± 2.3 7.5 (5.0 – 12.0)	3.1 ± 2.2 3.6 (0.1 – 5.9)	8.8 ± 2.7 10.0 (6.0 – 12.0)
Weight at baseline (kg)	68.2 ± 15.1 65.7 (52.0 – 90.7)	71.7 ± 12.7 70.2 (56.8 – 88.2)	21.2 ± 7.9 20.6 (11.4 – 35.4)	10.6 ± 5.2 12.2 (2.9 – 17.1)	33.8 ± 18.5 29.0 (18.4 – 62.6)
t <sub>last</sub> (h)	24.0 ± 0.02 24.0 (24.0 – 24.1)	24.0 ± 0.05 24.0 (23.9 – 24.1)	48.0 ± 0.05 48.0 (48.0 – 48.1)	48.1 ± 0.1 48.0 (47.9 – 48.3)	48.1 ± 0.05 48.1 (48.0 – 48.1)
t <sub>max</sub> (h)	12.0 ± 10.8 10.0 (0.0 – 24.1)	8.0 ± 8.3 6.0 (2.0 – 24.1)	20.8 ± 10.0 18.1 (11.9 – 32.2)	15.5 ± 10.3 12.0 (0.0 – 32.2)	16.0 ± 9.0 12.0 (12.0 – 32.0)
C <sub>max</sub> (U/L)	1241 ± 808 1078 (331 - 2739)	2529 ± 1507 1898 (1126 - 5199)	1781 ± 666 1757 (765 - 2866)	1576 ± 720 1282 (688 - 3316)	2413 ± 908 2297 (1106 - 3584)
AUC <sub>t</sub> (h*U/L)	19477 ± 6895 21707 (7220 - 25509)	44708 ± 24748 31219 (24967 - 80722)	75985 ± 28122 76282 (31576 - 120300)	58743 ± 29580 45827 (22330 - 138238)	104409 ± 40969 96657 (48422 - 160903)
C <sub>max,nor</sub> (U/L)/(U/kg)	4.7 ± 2.6 4.2 (1.4 – 9.3)	5.8 ± 3.2 4.5 (2.5 – 10.7)	1.0 ± 0.4 1.0 (0.42 – 1.6)	1.2 ± 0.87 0.80 (0.48 – 3.8)	0.9 ± 0.33 0.83 (0.40 – 1.3)
AUC <sub>t,nor</sub> (h*U/L)/(U/kg)	75.3 ± 25.2 81.7 (30.3 – 104)	102 ± 55.7 71.4 (54.3 – 181)	41.2 ± 15.6 40.5 (17.3 – 66.7)	43.6 ± 36.2 30.2 (13.3 – 160)	37.9 ± 14.9 34.4 (17.4 – 57.5)
Accumulation ratio <sup>c</sup>	5.3	6.2	3.8	2.5	2.3

<sup>a</sup>Mean ± S.D., <sup>b</sup>Median (Range), <sup>c</sup>Ratio values reflect the fold increase of AUC<sub>t,nor</sub> from Week 1 based on mean AUC<sub>t,nor</sub> values.

### 2.5.3.3 PK Parameter Estimates from Pop-PK Modeling

The Applicant developed a (b) (4) Pop-PK model to describe observed asfotase alfa PK following both IV and SC dosing. The typical value of clearance (CL/F) is 12.7 (95% CI: (b) (4)) L/day for a subject who has a body weight of 70 kg, is ADA-, and receives a product with a (b) (4) (Table 11). The inter-compartmental clearance (Q) was 52.9 (41.1 – 68.2) L/day, central volume of distribution (V<sub>2</sub>) was 4.55 (1.61 – 12.9) L, and peripheral volume of distribution (V<sub>3</sub>) was 44.6 (32.1 – 62.0) L. The estimated absolute bioavailability (F<sub>(b) (4) 40mg/mL</sub>) was 62.0 (58.7 – 65.5) % and the estimated absorption rate (K<sub>a (b) (4) 40mg/mL</sub>) was 0.662 (0.411 – 1.07) /day following SC administration of the (b) (4) scale product in 40 mg/mL.

The Pop-PK model included covariates to evaluate the effects of body weight, immunogenicity, product manufacturing scale, and formulation strength. See Sections 2.6.11, 2.6.4.4, 2.8.2, and 2.8.5 for more details.

**Table 11. Asfotase alfa Pop-PK model parameter estimates for a 70-kg individual**

(Source: Table 1 of Alexion’s response to Q8 of FDA IR dated January 26, 2015)

PK Parameter (Unit)*	NONMEM Parameter*	Estimate	95%CI**
V <sub>2,70kg</sub> (L)	exp(θ <sub>2</sub> )	4.55	(b) (4)
V <sub>3,70kg</sub> (L)	exp(θ <sub>3</sub> )	44.6	(b) (4)
Q <sub>70kg</sub> (L/day)	exp(θ <sub>4</sub> )	52.9	(b) (4)
k <sub>a (b) (4) 40mg/mL</sub> (day <sup>-1</sup> )	exp(θ <sub>5</sub> )	0.662	(b) (4)
F <sub>(b) (4) 40mg/mL</sub>	exp(θ <sub>6</sub> )	0.620	(b) (4)
ALAG (day)	exp(θ <sub>7</sub> )	0.140	(b) (4)
CL ~ ADA + /NAb-	exp(θ <sub>8</sub> )	1.13	(b) (4)
CL ~ ADA + /NAb+	exp(θ <sub>9</sub> )	1.22	(b) (4)
k <sub>a (b) (4) ~ Batchsize (b) (4)</sub>	exp(θ <sub>11</sub> )	0.881	(b) (4)
F <sub>(b) (4) ~ Batchsize (b) (4)</sub>	exp(θ <sub>12</sub> )	0.880	(b) (4)
CL ~ AllometricExponent	θ <sub>13</sub>	0.733	(b) (4)
k <sub>a,40mg/mL ~ FormulationStrength<sub>100mg/mL</sub></sub>	exp(θ <sub>14</sub> )	1.00	(b) (4)
F <sub>40mg/mL ~ FormulationStrength<sub>100mg/mL</sub></sub>	exp(θ <sub>15</sub> )	0.762	(b) (4)
IIVvar CL (ω <sub>CL</sub> <sup>2</sup> )	Ω <sub>1,1</sub> (η <sub>1</sub> )	0.195 (%CV=46.5)	(b) (4)
IIVcov CL, V <sub>2</sub> (ω <sub>CL</sub> ω <sub>V<sub>2</sub></sub> )	Ω <sub>2,1</sub>	-0.0601	(b) (4)
IIVvar V <sub>2</sub> (ω <sub>V<sub>2</sub></sub> <sup>2</sup> )	Ω <sub>2,2</sub> (η <sub>2</sub> )	0.549 (%CV=85.5)	(b) (4)
IIVcov CL, V <sub>3</sub> (ω <sub>CL</sub> ω <sub>V<sub>3</sub></sub> )	Ω <sub>3,1</sub>	0.0344	(b) (4)
IIVcov V <sub>2</sub> , V <sub>3</sub> (ω <sub>V<sub>2</sub></sub> ω <sub>V<sub>3</sub></sub> )	Ω <sub>3,2</sub>	0.280	(b) (4)
IIVvar V <sub>3</sub> (ω <sub>V<sub>3</sub></sub> <sup>2</sup> )	Ω <sub>3,3</sub> (η <sub>3</sub> )	0.168 (%CV=42.7)	(b) (4)
IIVcov CL, k <sub>a</sub> (ω <sub>CL</sub> ω <sub>k<sub>a</sub></sub> )	Ω <sub>4,1</sub>	-0.139	(b) (4)
IIVcov V <sub>2</sub> , k <sub>a</sub> (ω <sub>V<sub>2</sub></sub> ω <sub>k<sub>a</sub></sub> )	Ω <sub>4,2</sub>	0.0493	(b) (4)
IIVcov V <sub>3</sub> , k <sub>a</sub> (ω <sub>V<sub>3</sub></sub> ω <sub>k<sub>a</sub></sub> )	Ω <sub>4,3</sub>	-0.0585	(b) (4)
IIVvar k <sub>a</sub> (ω <sub>k<sub>a</sub></sub> <sup>2</sup> )	Ω <sub>4,4</sub> (η <sub>4</sub> )	0.801 (%CV=111)	(b) (4)
Res <sub>additive</sub> (σ <sub>additive</sub> )	Σ <sub>1,1,additive</sub> (ε <sub>1</sub> )	0.136 (SD=0.4)	(b) (4)

\* Estimate of θ modeling in the log domain were exponentiated and are reported in the table.

\*\* 95% CI derived from standard errors obtained from the Covariance step in NONMEM. For the 2 batch size and formulation strength-related parameters, a 90% CI is reported.

### 2.5.4 How does the PK of asfotase alfa in healthy adults compare to that in patients with the target disease?

Based on the preliminary PK data in healthy subjects and data in a small number of adult HPP patients,  $C_{max}$  and  $t_{1/2}$  appeared relatively similar between the two studied samples but AUC exposure appeared to be higher in healthy subjects than in HPP patients.

Table 12 below contained data in healthy subjects. Mean  $C_{max}$  value was (b) (4)

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**Table 12. Preliminary mean (SD) asfotase alfa PK parameters in healthy volunteers received 2 mg/kg SC dose of asfotase alfa in Study AA-HV-104\***

(Source: Table 16 of Alexion's response to FDA IR dated January 26, 2015)

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The Applicant conducted a healthy volunteer study (Study AA-HV-104) in Canada to evaluate the biocomparability of a (b) (4) formulation (b) (4) with the reference formulation (b) (4) the to-be-marketed product/formulation being used in the clinical studies supporting the current BLA submission). (b) (4)

The Applicant provided the preliminary PK information for both (b) (4) formation product and the reference Process A formulation product in response to the Agency's Information IR dated January 26, 2015.

The results presented in Table 12 are considered preliminary because the final Clinical Study Report is pending.

**2.5.5 What is the inter-subject variability of the PK parameters in patients with the target disease?**

The inter-subject variability for the observed  $AUC_t$  over the dosing interval after multiple SC dosing of asfotase alfa at different dose levels in HPP patients ranged from 35.4% to 55.4% (Table 10). The inter-subject variability for the estimated Pop-PK parameters CL,  $V_2$ ,  $V_3$ , and  $k_a$  were 46.5, 85.5, 42.7, and 111%, respectively (Table 11).

**2.5.6 What are the characteristics of drug absorption, distribution, metabolism, and elimination?**

### 2.5.6.1 Absorption

As described in Section 2.5.2, the observed median  $t_{max}$  ranged from 1 to 2 days and the absolute bioavailability ranged from (b) (4) % following weekly SC administrations of asfotase alfa 2 mg/kg in Study ENB-001-08 (Table 7). The mean  $\pm$  SD observed  $C_{max}$  and  $AUC_t$  at Week 4 were  $1020 \pm 326$  U/L and  $136109 \pm 41875$  U\*h/L, respectively for the 2 mg/kg dose group.

### 2.5.6.2 Distribution

The Pop-PK estimates central ( $V_{2,70kg}$ ) and peripheral ( $V_{3,70kg}$ ) volumes of distribution (and 95% CI) were  $4.55$  (b) (4) L and  $44.6$  (b) (4) L, respectively. These results indicate that asfotase alfa is initially distributed in the intra-vascular space and then distributes to the extra-vascular space reflecting the ability of asfotase alfa to partition into tissues.

### 2.5.6.3 Metabolism

No studies on the metabolism of asfotase alfa have been performed in humans or in animals. Asfotase alfa is a therapeutic protein which is expected to be degraded via peptide hydrolysis to amino acids.

### 2.5.6.4 Elimination

The excretion of unchanged asfotase alfa in urine is not expected as asfotase alfa has a molecular weight of (b) (4). The typical value of CL is 12.7 (95% CI: (b) (4)) L/day for a subject who has a body weight of 70 kg, is ADA-, (b) (4). The mean  $t_{1/2}$  was approximately 130 hours for both HPP patients and healthy subjects (Table 7 and Table 12).

### ***2.5.7 Based on PK parameters, what is the degree of the proportionality of the dose-concentration relationship?***

Based on the mean PK parameters following the SC administration of the first dose, the PK of asfotase alfa appeared to be dose proportional from 0.3 mg/kg to 3 mg/kg (see Section 2.5.3.1).

Based on the Pop-PK modeling analysis, dose proportionality was inferred because the linear PK model adequately described the observed concentration-time data obtained in patients who received doses up to 28 mg/kg/week.

### ***2.5.8 How do the PK parameters change with time following chronic dosing?***

In Study ENB-009-10, mean  $C_{max}$  and  $AUC_t$  values for the 0.3 mg/kg and the 0.5 mg/kg appeared not to be different between Week 3 and Week 24 (Table 13), suggesting that asfotase alfa PK did not change after chronic dosing.

**Table 13. Summary of PK parameters following multiple dosing of asfotase alfa in Study ENB-009-10**

(Source data submitted in Alexion’s response to IR dated January 26, 2015)

Dose	0.3 mg/kg (N = 7)			0.5 mg/kg (N = 6)		
Week	3	12	24	3	12	24
n	6	6	4	6	5	4
(b) (4)						
Age (years)	43.7 ± 23.4 51.0 (14.0 – 66.0)	43.7 ± 23.4 51.0 (14.0 – 66.0)	47.8 ± 23.2 54.5 (16.0 – 66.0)	48.5 ± 16.5 55.0 (15.0 – 57.0)	47.2 ± 18.1 55.0 (15.0 – 57.0)	55.3 ± 1.7 55.0 (53.0 – 57.0)
Weight at baseline (kg)	68.2 ± 15.1 65.7 (52.0 – 90.7)	68.2 ± 15.1 65.7 (52.0 – 90.7)	68.5 ± 17.3 65.7 (52.0 – 90.7)	71.7 ± 12.7 70.2 (56.8 – 88.2)	72.2 ± 14.1 71.3 (56.8 – 88.2)	75.2 ± 14.3 78.0 (56.8 – 88.2)
t <sub>last</sub> (h)	24.0 ± 0.02 24.0 (24.0 – 24.1)	24.0 ± 0.01 24.0 (23.9 – 24.2)	24.1 ± 0.08 24.0 (24.0 – 24.2)	24.0 ± 0.05 24.0 (23.9 – 24.1)	24.0 ± 0.07 24.0 (23.9 – 24.1)	24.1 ± 0.06 24.1 (24.0 – 24.1)
t <sub>max</sub> (h)	12.0 ± 10.8 10.0 (0.0 – 24.1)	9.7 ± 11.1 3.1 (1.9 – 24.0)	6.0 ± 6.7 3.1 (2.0 – 16.0)	8.0 ± 8.3 6.0 (2.0 – 24.1)	14.0 ± 9.8 12.0 (2.0 – 24.1)	8.5 ± 10.9 5.0 (0.0 – 24.1)
C <sub>max</sub> (U/L)	1241 ± 808 1078 (331 - 2739)	972 ± 398 990 (496 - 1435)	779 ± 228 749 (571 - 1044)	2529 ± 1507 1898 (1126 - 5199)	1915 ± 591 1812 (1233 - 2866)	1937 ± 998 1955 (913 - 2927)
AUC <sub>t</sub> (h*U/L)	19477 ± 6895 21707 (7220 - 25509)	20720 ± 9920 20535 (8023 - 32445)	16634 ± 5264 15437 (12364 - 23295)	44708 ± 24748 31219 (24967 - 80722)	40340 ± 14226 40113 (23287 - 61847)	41226 ± 23360 39865 (18922 - 66252)
C <sub>max,nor</sub> (U/L)/(U/kg)	4.7 ± 2.6 4.2 (1.4 – 9.3)	3.8 ± 1.8 3.7 (1.6 – 6.0)	3.0 ± 1.0 3.1 (2.0 – 3.9)	5.8 ± 3.2 4.5 (2.5 – 10.7)	4.3 ± 1.2 4.6 (2.7 – 6.0)	4.6 ± 2.6 4.6 (2.0 – 7.4)
AUC <sub>t,nor</sub> (h*U/L)/(U/kg)	75.3 ± 25.2 81.7 (30.3 – 104)	82.0 ± 43.7 77.2 (25.4 – 136)	63.6 ± 21.6 62.6 (43.2 – 86.2)	102 ± 55.7 71.4 (54.3 – 181)	90.6 ± 29.3 85.7 (51.1 – 129)	98.9 ± 60.5 93.2 (42.0 – 167)

<sup>a</sup>Mean ± S.D., <sup>b</sup>Median (Range).

## 2.6 Intrinsic Factors

### 2.6.1 *What are the major intrinsic factors responsible for the inter-subject variability in exposure in patients with the target disease?*

Body weight and immunogenicity are the major intrinsic factors influencing systemic exposure in patients with HPP. Effect of body weight is discussed below and immunogenicity impact is discussed in section 2.6.4.4.

To identify intrinsic factors that could contribute to the pharmacokinetic variability, the Applicant included two components in the covariate modeling step: a pre-specified covariate model for inferential purposes and an exploratory, hypothesis-generating, covariate model evaluated post hoc.

Under the exploratory covariate analysis, relationships between asfotase alfa CL and renal function as measured by estimated glomerular filtration rate (eGFR), liver function as measured by ALT and AST, and race were evaluated. None of these factors were found to be significant covariates to the model.

#### 2.6.1.1 Effects of Body Weight on CL and V

Results of Pop-PK modeling analysis identified that body weight affected asfotase alfa CL and volume of distribution parameters. The volume of distribution parameters increased linearly with body weight (i.e., with an allometric scaling exponent value of 1), and CL increased with body weight following an allometric scaling factor of 0.733 (95% CI: (b) (4)). Based on these results, it is expected that PK exposures will increase with body weight when doses are administered based on body weight (mg/kg) as in the case of the asfotase alfa.

Figure 8 illustrates the effect of body weight on systemic exposure using simulated mean (90% CI) value of asfotase alfa average concentration at steady state ( $C_{avg,ss}$ ) across subjects with different body weights. In subjects who weigh 4.5 kg to 75 kg and are ADA-negative, the simulated  $C_{avg,ss}$  increases from 1636 to 3486 U/L when given 40 mg/mL formulation of the (b) (4) product (b) (4) at 2 mg/kg three times weekly.

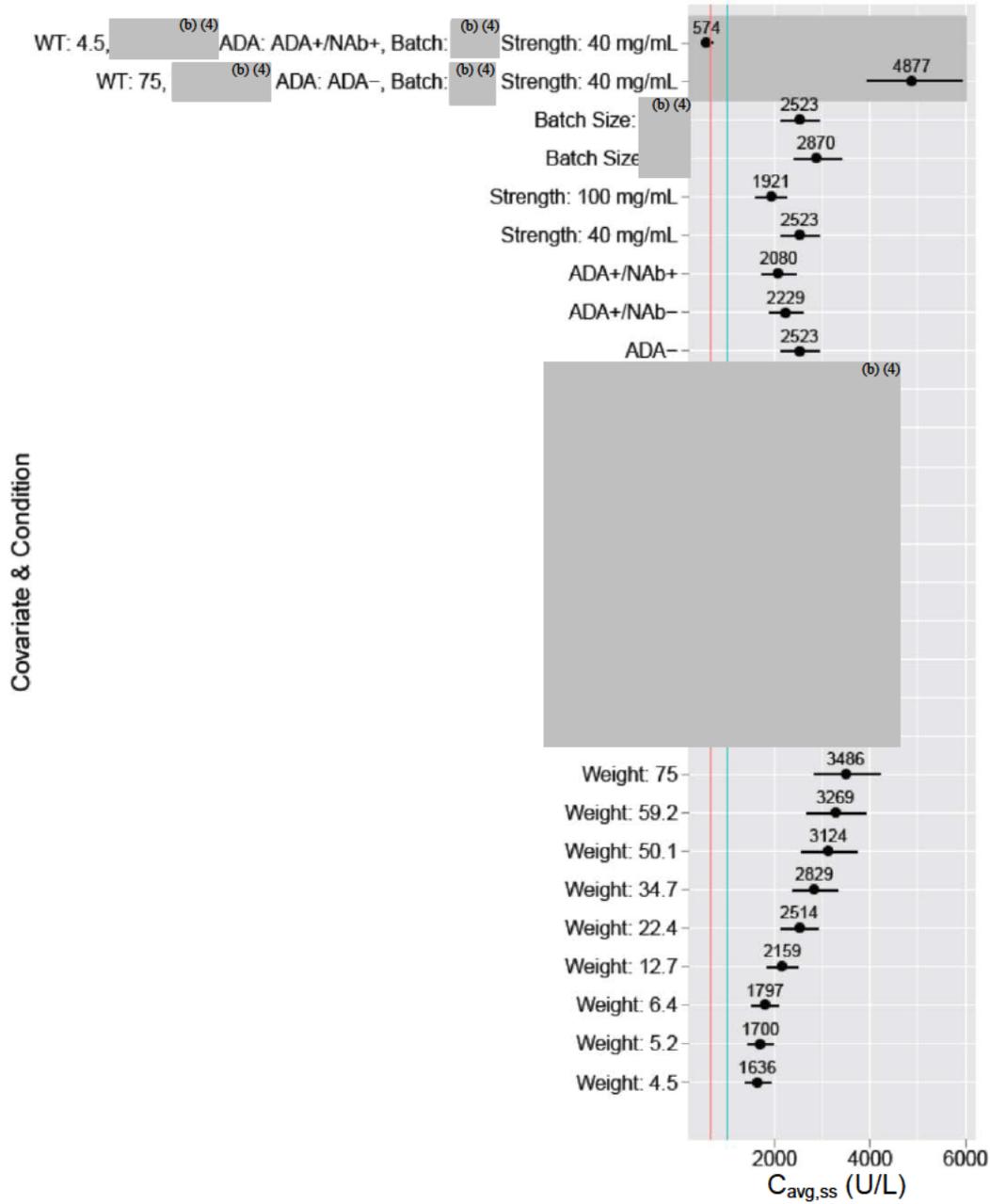
### 2.6.2 *Based upon what is known about E-R relationships in the target population and their variability, what dosage regimen adjustments are recommended for each group?*

No dosing adjustments are recommended for the following patient characteristics.

#### 2.6.2.1 Disease Phenotype

Dose adjustments are not necessary because the simulation showed overlapping E-R relationships between the infantile- and juvenile-onset subjects (Figure 5), suggesting that dosing with one dosing regimen of 6 mg/kg/week is reasonable.

**Figure 8. Impact of different covariates and their combination on simulated  $C_{avg,ss}$**   
 (Source: Figure 4 of Alexion’s response to Q8 of FDA IR dated January 26, 2015)



$C_{avg,ss}$  values are based on a regimen of 2 mg/kg administered three times per week. Dose activity is assumed to be 990 U/mg. Covariates were fixed at the following values, except when being tested: (b) (4) weight, 22.7 kg; anti-drug and neutralizing antibodies, negative; batch size (b) (4) and formulation strength 40 mg/mL. The blue and red lines depict efficacious exposure levels from nonclinical efficacy mouse studies that defined the targeted exposure thresholds for clinical effect.

#### 2.6.2.2 Age

No dose adjustments are needed because age is correlated with body weight and the dosing is weight-based.

#### 2.6.2.3 Body Weight

The proposed dosing is based on body weight.

#### 2.6.2.4 Elderly

There is limited information of asfotase alfa PK in elderly; only one subject > 65 years old was enrolled in clinical trial. Hence, no dose adjustments are recommended for this population.

#### 2.6.2.5 Pediatric Patients

In the Applicant's simulation for the proposed dose regimens i.e., 6 mg/kg/week (Figure 8), exposures in the youngest patients appeared lower than those for the remainder of the population. However, based on the distribution of  $C_{avg}$  for the population by age (Figure 6, also see Pharmacometric review), the mean values of  $C_{avg}$  for all different patient age groups were above 2000 U/L and the exposures in the youngest patients (< 6 months) were actually higher than majority of the population. As such, no dose adjustments are recommended.

#### 2.6.2.6 Race

Based on the simulated  $C_{avg,ss}$ , it was estimated that Asians had a clearance 68.8% of the estimate in non-Asians (See Applicant's Population PKPD Report). However, due to the wide 95% CI of the estimate (b) (4) and the limited number of Asian subjects in the Pop-PK dataset (n = 6), the finding remains to be confirmed, and therefore, no dose adjustments are recommended.

#### 2.6.2.7 Sex

No dose adjustments are necessary because sex was not a significant covariate of the Pop-PK model.

#### 2.6.2.7 Renal and Hepatic Impairment

Renal function by eGFR and hepatic function by liver transaminase levels were not found to influence asfotase afa CL when evaluated within the Pop-PK framework. Dose adjustments are not needed.

### ***2.6.3 Does genetic variation impact exposure and/or response?***

HPP is a rare inherited disorder caused by mutations in the alkaline phosphatase liver-type (*ALPL*) gene, which encodes the TNSALP enzyme, that can be transmitted in an autosomal dominant or recessive manner. With the exception of a few more common mutations that have

been identified in certain populations (such as c.1559delT, c.979T>C, c.571G>A, c.1001G>A, c.1133A>T), the majority of mutations are distributed throughout the 12 exons of the gene and contribute to a large number of compound heterozygotes. As of May 6, 2015, at least 300 mutations have been reported with the most common type being missense (75%) followed by small deletions (11%), splicing (9%), nonsense (4%), small insertions (2%), large deletions (1%), insertion/deletions (<1%), and regulatory (<1%) [[http://www.sesep.uvsq.fr/03\\_hypo\\_mutations.php](http://www.sesep.uvsq.fr/03_hypo_mutations.php)].

Mutations in *ALPL* were assessed in 102 patients with infantile- and juvenile-onset HPP in Studies ENB-002-08, ENB-010-10, ENB-006-09, and ENB-009-10. Mutations were provided for 84% (59/70) of infantile-onset patients, corresponding to 100% (11/11) of patients in Study ENB-002-08 and 81% (48/59) of patients in Study ENB-010-10. One patient in Study ENB-010-10 (Patient ENB-010-010-16-06) did not have a disease-causing mutation identified. The 11 patients from Study ENB-010-10 who did not have mutations provided either had *ALPL* analysis from a certified laboratory available but did not provide the results (n = 10) or mutations were not assessed (n = 1). Of the infantile-onset patients with genetic data, the most common genotypes found in >1 patient were c.571G>A/c.1001G>A (n = 5), c.571G>A/c.1133A>T (n = 4), c.1001G>A/NA (n = 3), c.1559DEL/c.1559DEL (n = 2), c.293C>T/c.293C>T (n = 2), and c.571G>A/c.212G>A (n = 2), with the remaining 41 patients having unique genotypes. Alleles identified in infant-onset patients were missense (84%), small deletions (10%), insertion/deletions (3%), nonsense (2%), and splice-site mutations (2%).

In the juvenile-onset patients, mutations in *ALPL* were assessed for all patients in Studies ENB-006-09 (N = 13) and ENB-009-10 (N = 19). For 1 patient in Study ENB-006-09 (Patient ENB-006-09-02-03), a disease-causing mutation was not identified. The most common genotypes found in >1 juvenile-onset patient were c.571G>A/c.1001G>A (n = 4), c.526G>A/c.881A>C (n = 3), c.571G>A/c.550C>T (n = 2), c.1133A>T/NA (n = 2), c.526G>A/IVS6+1G>A (n = 2), and c.571G>A/c.1250A>G (n = 2), with the remaining 18 patients having unique genotypes. Alleles identified in juvenile-onset patients were missense (93%), small deletions (2%), and splice-site mutations (5%).

### **Reviewer's Comments:**

*The applicant did not assess the potential impact of genetic variation on exposure, immunogenicity, or response for asfotase alfa in HPP patients. Given the large number of distinct ALPL genotypes reported, the impact of ALPL genotype on the exposure and/or response to asfotase alfa cannot be reliably assessed at this time. The reviewer agrees with the applicant's approach.*

### **2.6.4 Immunogenicity**

#### **2.6.4.1 What data are available for the assessment of the incidence of immunogenicity and the impact of immunogenicity on PK, efficacy, and safety?**

In the initial BLA submission, the Applicant provided immunogenicity data for 73 HPP patients over a period of time (up to 3 years) as of data cutoff date of November 22, 2013. Upon IRs from Agency including Clinical IR dated March 10, 2015 and Clinical Pharmacology IR dated

March 18, 2015, the Applicant provided updated information on the impact of immunogenicity on safety, using data from a total of 102 patients with HPP as of data cutoff date of November, 2014.

2.6.4.2 What is the incidence of the formation of the anti-drug antibodies (ADA)? What is the time course of the development of ADA?

For the 71 of the 73 subjects with post baseline immunogenicity data, 57 (80%) subjects had at least one measurement that was ADA+ during treatment and were considered to have ADA+ status. Fourteen (20%) subjects had no samples that were ADA+ and were considered to have ADA- status (Table 14). After the initial occurrence of ADA+ sample, the subsequent immunogenicity samples remained ADA+ in 31 (54%) of the 57 ADA+ subjects, while 26 (46%) subjects had at least one ADA- sample. In other words, ADA developed and sustained in 31 of the 71 (44%) subjects.

Figure 9 depicts the time course of the development of ADA. Low ADA titer was observed in the majority of the patients, with a few patients having ADA titer values >500 for a prolonged period. Also, a number of patients had samples fluctuate between ADA+ and ADA- with low titer values ( $\leq 64$ ) as seen in the inset of Figure 9.

**Table 14. Incidence of ADA and NAb in 71 subjects with post baseline immunogenicity data**  
(Source: Table 23 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

Group	Category	Patients % (N)	Samples % (n)		Total Samples
			ADA+	ADA-	
ADA status	ADA-	20% (14 of 71)	0	100% (118)	118
	ADA+	80% (57 of 71)	66% (368)	34% (187)	555
	ADA+ Only	54% (31 of 57)	84% (215)	16% (42)	257
	ADA+/- Mix	46% (26 of 57)	51% (153)	49% (145)	298
ADA+/ NAb status	NAb+	44% (25 of 57)	19% (69 of 368)	0	368
	NAb-	56% (32 of 57)	81% (299 of 368)	0	368

ADA status = ADA+: at least one positive ADA sample

ADA status = ADA-: no positive ADA sample

ADA+ Only subcategory: all samples were ADA+ after the first occurrence of ADA+ sample

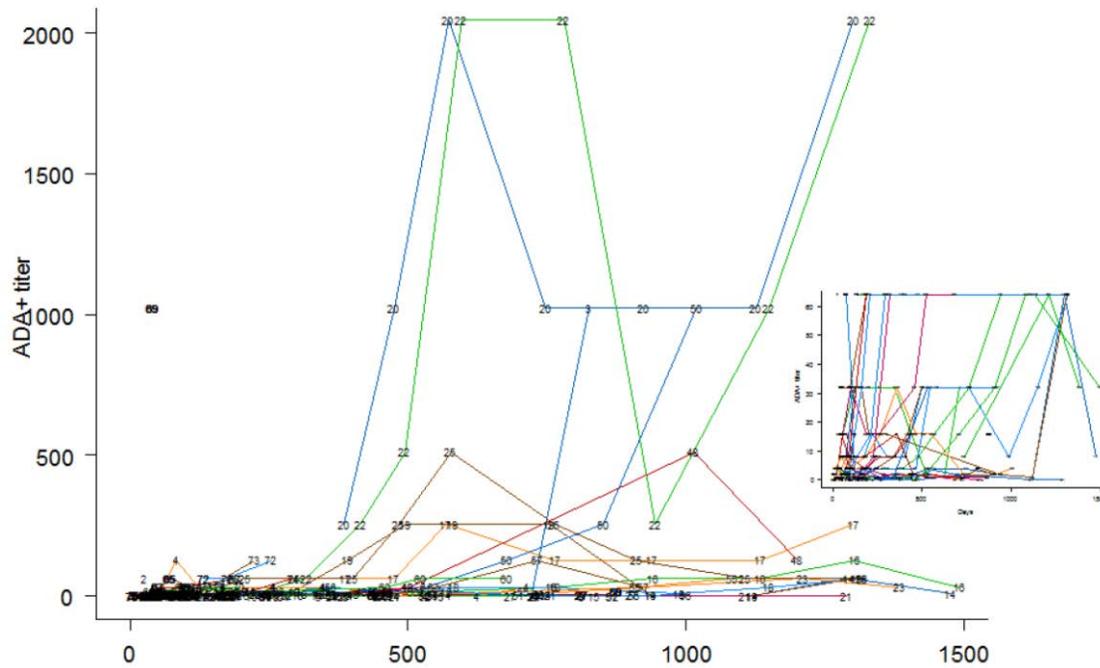
ADA+/- Mix subcategory: at least one ADA sample were negative after the first occurrence of ADA+ sample

NAb+: at least one sample positive for NAb

NAb-: no sample positive for NAb

**Figure 9. Development of ADA over time**

(Source: Figure 19 of Module 2.7.2 Summary of Clinical Pharmacology Studies)

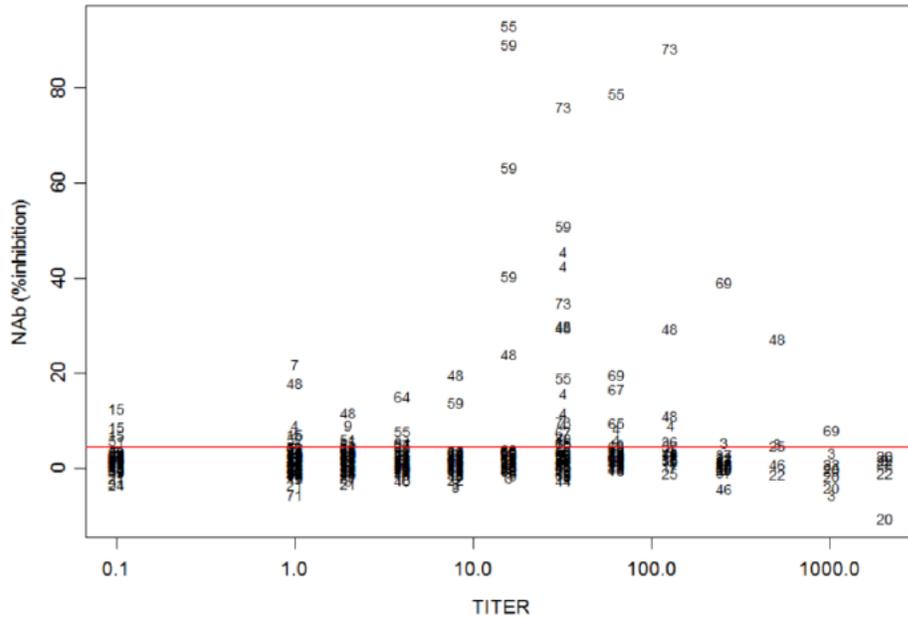


2.6.4.3 Do the antibodies to asfotase alfa have neutralizing capability and what is the incidence (rate) of the formation of the neutralizing antibodies (NAb)? Is there correlation between ADA titer and NAb % inhibition?

Yes, the antibodies to asfotase alfa have neutralizing capability. Among the 57 subjects with ADA, 25 (44%) subjects had at least one NAb positive sample and 32 (56%) subjects were NAb- (Table 14). In the 673 samples collected throughout the development program and tested for ADA and NAb status, 19% were found to be ADA+/NAb+.

There appeared no correlation between ADA titer and NAb % inhibition (Figure 10).

**Figure 10. Correlation between ADA titer and NAb % inhibition**  
 (Source: Figure 20 of Module 2.7.2 Summary of Clinical Pharmacology Studies)



The 0.1 label on the x-axis represents PK samples that were ADA+ with a 0 titer value (a titer of 0 corresponds to minimum required dilution of the titer assay or MRD); the red horizontal line is the NAb threshold of (b) (4)

2.6.4.4 Does the immunogenicity affect the PK of asfotaes alfa?

Yes, immunogenicity had a negative impact on the PK of asfotase alfa. Based on a subset of subjects who have available AUC data at Week 6, the asfotase alfa exposure in ADA- subjects was approximately 2-fold greater than the exposure in ADA+ subjects.

The Clinical Pharmacology reviewer assessed the impact of immunogenicity on PK using dose-normalized  $AUC_t$  over a dosing interval (i.e. 48-hour  $AUC_{t,nor}$ ) obtained from NCA at Week 6 (assumed to be steady state) in 31 pediatric patients with HPP in Studies ENB-006-09 and ENB-010-10.  $AUC_{t,nor}$  was calculated by dividing the AUC value by the total dose in activity units (i.e., U) which was obtained by multiplying the administered dose amount in mg/kg by the specific activity of the batch of the drug used (U/mg). The median (range) age of the subjects was 5 (0.1 – 12) years old and the median weight was 13.8 (2.9 – 62.6) kg. (b) (4)

(b) (4) All PK concentrations were analyzed by the method from (b) (4) method.

A general linear model was used to assess the effect of ADA positivity on  $AUC_{t,nor}$  at Week 6 when PK was assessed, with age, (b) (4) product scale, and formulation strength evaluated as covariates in the model. Results showed that (b) (4)

(b) (4)

The difference in AUC values was also assessed by the Mann-Whitney U test. The median (range) of  $AUC_{t,nor}$  was 41.4 (20.1 – 160) (h\*U/L)/(U/kg) in ADA- subjects and was higher than the median value of 27.5 (9.3 – 89.9) (h\*U/L)/(U/kg) in ADA+ subjects ( $p = 0.024$ ) (Figure 11).

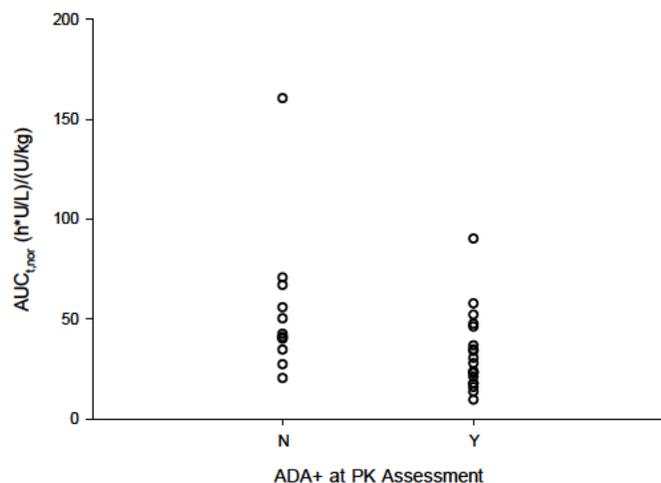
The 1.5- to 2-fold difference in  $AUC_{t,nor}$  observed by NCA method was larger than the difference (< 20%) estimated by the Pop-PK analysis (see below)

**Table 15. Comparison of  $AUC_{t,nor}$  between ADA- and ADA+ subjects at Week 6**

	$AUC_{t,nor}$ (h*U/L)/(U/kg)		
	ADA- (n = 12)	ADA+ (n = 19)	Ratio* of ADA-/ADA+ <sup>(b) (4)</sup>
Least squares means (SE)			
Median (range)	41.4 (20.1 - 160)	27.5 (9.3 - 89.9)	1.5

\*Ratio calculated based on the mean or median value for each group.

**Figure 11.  $AUC_{t,nor}$  between ADA+ and ADA- subjects at Week 6**



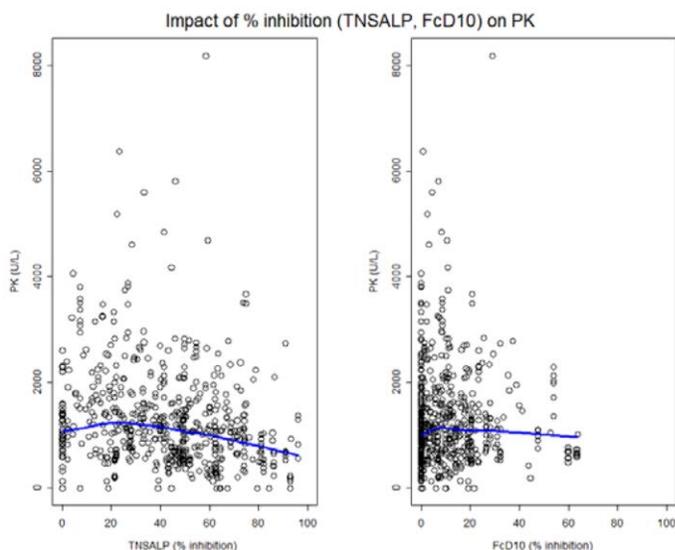
The Applicant assessed the impact of immunogenicity on PK by implementing immunogenicity as a time-dependent categorical covariate; immunogenicity sample was assigned to one of 3 possible subgroups: ADA- (no NAb measured), ADA+/NAb+, and ADA+/NAb-. The model parameter estimates showed the impact of ADA+/NAb- state to have 11% (95 % CI: <sup>(b) (4)</sup>) greater CL compared to ADA- state and ADA+/NAb+ state to have 21% (95% CI: 8 <sup>(b) (4)</sup>) greater CL compared to ADA- state.

The Applicant further evaluated the impact of immunogenicity via simulation of asfotase alfa  $C_{avg,ss}$  using a regimen of 2 mg/kg SC 3x/week. Results showed that  $C_{avg,ss}$  was lower in ADA+/NAb- or ADA+/NAb+, but the 90% CI of  $C_{avg,ss}$  showed significant overlap (Figure 8). Therefore, the Applicant considered the immunogenicity response for asfotase alfa is considered low based on the impact of immunogenicity on PK of asfotase alfa. However, the Applicant’s analysis appeared to have under-estimated the impact of immunogenicity when compared to the results of the reviewer’s independent analysis.

The Applicant also assessed the impact of % inhibition by TNSALP and FcD10 on asfotase alfa PK. No apparent trend was observed between asfotase alfa concentration and % inhibition by TNSALP and FcD10 (Figure 12).

**Figure 12. Asfotase alfa concentration level vs. % inhibition by TNSALP (left panel) and FcD10 (right panel)**

(Source: Figure 2 of Applicant's response to FDA IR dated March 18, 2015)



#### 2.6.4.5 Does the immunogenicity affect the PD of asfotase alfa?

Using XY plots of response vs. % inhibition, the Applicant assessed visually the impact of % inhibition by TNSALP and FcD10 on PD measurements including RSS knee, RSS wrist, RGI-C, BOT-2, and 6MWT%pred. No clear trends or associations were observed between different responses and % inhibition by TNSALP and FcD10. For the XY plots of these PD measurements, refer to Figures 2, 3, and 5 of the Applicant's response to FDA IR dated March 18, 2015.

#### 2.6.4.6 What is the impact of immunogenicity on clinical efficacy (i.e., survival in infantile-onset HPP patients and MPOMA-G score in juvenile-onset HPP patients)?

The impact of immunogenicity on clinical efficacy cannot be evaluated adequately for both the infantile-onset HPP patients and the juvenile-onset HPP patients.

For the infantile-onset subjects, the overall survival was summarized by immunogenicity status and presented in Table 16. Whether immunogenicity impacted the overall survival could not be reliably determined because the analysis was limited by the number of subjects who died.

**Table 16. Comparison of immunogenicity data for infantile-onset HPP patients who were censored at the latest visit or latest dose date with those who died**

	Censored (n = 60)	Death (n = 7)
ADA+ Status		
Yes	44 (73.3 %)	4 (57.1%)
No	16 (26.7 %)	3 (42.9%)
Continuously ADA+		
Yes	31 (51.7 %),	3 (42.9%)
No	29 (48.3%)	4 (57.1%)
NAb+ Status		
Yes	22 (36.7%)	4 (57.1%)
No	38 (63.3%)	3 (42.9%)
Titer in ADA+ Subjects		
Low Titer, ≤ 128	7/44 (15.9 %)	2/4 (50%)
High Titer, > 128	37/44 (84.1 %)	2/4 (50%)

For the juvenile-onset HPP subjects, no assessment was performed by the Clinical Pharmacology reviewer because of the small number of subjects with MPOMA-G score (n = 8) and the limitations of the quality of the 6MWT video being used for the gait assessment.

#### 2.6.4.7 What is the impact of immunogenicity on clinical safety?

Immunogenicity appeared to have an impact on the rate of ISR and ectopic calcification; ADA+ subjects had a slightly higher rate of the two adverse events. Because of the small number of subjects and the short duration of the clinical trials, long term immunogenicity and safety assessments are warranted to provide further insight into the impact of immunogenicity on safety.

The impact of immunogenicity on safety was evaluated using an updated data set of 102 patients with HPP as of data cutoff date of November, 2014 (see Section 2.6.4.1). The analysis focused on the impact of ADA positivity and % inhibition by domain (TNSAP and FcD10) on the following three adverse events: ISR, IAR, and ectopic calcification.

Immunogenicity appeared to have an impact on the rate of ISR and ectopic calcification as shown in Table 17. The incidence of ISR was 24.5% higher in ADA+ than in ADA- subjects. However, ISR may be of little clinical importance in HPP patients who suffer significant morbidity from the disease. Of note, the incidence of ISR was relatively high at 50% even in ADA- subjects, suggesting other factors might have contributed to the reactions.

The incidence of ectopic calcification was approximately 10% higher in ADA+ than in ADA- subjects. The clinical significance of this impact of this finding is unclear because ectopic calcification can be a manifestation of the disease, except for deposit in the cornea and worsening of kidney stone.

The rate of IAR was similar between ADA+ and ADA- subjects.

**Table 17. Impact of immunogenicity on safety**

Adverse Event	ADA-	ADA+
Injection site reactions	12/24 (50%)	58/78 (74.5%)
Infusion/injection associated reactions	5/24 (20.8%)	11/78 (14.1%)
Ectopic calcification	4/24 (16.7%)	21/78 (26.9%)

The impact of immunogenicity on safety was also explored by quartile of the % inhibition by domains. There were no consistent trends between the incidence of the aforementioned AEs by the % inhibition by TNSALP (Table 18, Table 19, and Table 20) or the % inhibition by FcD10 (Table 21, Table 22, and Table 23).

**Table 18. Incidence of ISR by quartiles of % inhibition of TNSALP**

(Source: Table 1 of Applicant's response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
TNSALP % Range	NA	[0 – 33.4]	[33.4 – 56.4]	[56.4 – 73.9]	[73.9 – 96.0]
patients with ISR in quartile / total patients in quartile	12 / 24	12 / 19	17 / 20	16 / 20	13 / 19
proportion of affected patients (95 % CI)	0.50 (0.29, 0.71)	0.63 (0.38, 0.84)	0.85 (0.62, 0.97)	0.80 (0.56, 0.94)	0.68 (0.43, 0.87)
mean (SD) rate of incidence	3.1 (3.4) <sup>1</sup>	6.4 (7.0)	7.6 (13.3)	9.4 (9.0)	15.8 (21.6)
median rate of incidence	1.8 <sup>1</sup>	4.6	3.4	5.3	5.7

<sup>1</sup> Patient ENB-010-10-16-11 excluded due to treatment duration of less than one week

Rate of incidence calculated by number of events/duration of treatment in years;

95% CI for proportion of affected patients determined by Clopper-Pearson method

70/102 (69%) patients had an ISR adverse event, of which 58 patients were ADA+

**Table 19. Incidence of IAR by quartiles of % inhibition of TNSALP**

(Source: Table 2 of Applicant's response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
TNSALP % Range	NA	[0 – 33.4]	[33.4 – 56.4]	[56.4 – 73.9]	[73.9 – 96.0]
patients with IAR in quartile / total patients in quartile	5 / 24	2 / 19	1 / 20	4 / 20	4 / 19
proportion of affected patients (95 % CI)	0.21 (0.07, 0.42)	0.11 (0.01, 0.33)	0.05 (0.00, 0.25)	0.20 (0.06, 0.44)	0.21 (0.06, 0.46)
mean (SD) rate of incidence	4.6 (5.2) <sup>1</sup>	1.2 (0.2)	2.7 <sup>2</sup>	0.5 (0.3)	1.6 (1.5)
median rate of incidence	2.9 <sup>1</sup>	1.2	2.7 <sup>2</sup>	0.5	1.3

<sup>1</sup> Patient ENB-002-08-03-01 was excluded from rate of incidence calculations due to only one dose administered

<sup>2</sup> Reported value is not mean or median as N=1

Rate of incidence calculated by number of events/duration of treatment in years;

95% CI for proportion of affected patients determined by Clopper-Pearson method

16/102 (16%) patients had an IAR adverse event, of which 11 patients were ADA+

**Table 20. Incidence of ectopic calcification by quartiles of % inhibition of TNSALP**

(Source: Table 3 of Applicant's response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
TNSALP % Range	NA	[0 – 33.4]	[33.4 – 56.4]	[56.4 – 73.9]	[73.9 – 96.0]
patients with Ectopic Calcification in quartile / total patients in quartile	4 / 24	4 / 19	9 / 20	3 / 20	5 / 19
proportion of affected patients (95 % CI)	0.17 (0.05, 0.37)	0.21 (0.06, 0.46)	0.45 (0.23, 0.68)	0.15 (0.03, 0.38)	0.26 (0.09, 0.51)
mean (SD) rate of incidence	1.3 (0.7)	0.4 (0.2)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)
median rate of incidence	1.3	0.4	0.3	0.2	0.3

Rate of incidence calculated by number of events/duration of treatment in years;  
95% CI for proportion of affected patients determined by Clopper-Pearson method  
25/102 (25%) patients had an Ectopic Calcification adverse event, of which 21 patients were ADA+

**Table 21. Incidence of ISR by quartiles of % inhibition of FcD10**

(Source: Table 4 of Applicant's response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
FcD10% Range	NA	[0 – 9.09]	[9.09 – 19.4]	[19.4 – 38.7]	[38.7 – 96.6]
patients with ISR in quartile / total patients in quartile	12 / 24	10 / 19	17 / 20	14 / 20	17 / 19
proportion of affected patients (95 % CI)	0.50 (0.29, 0.71)	0.53 (0.29, 0.76)	0.85 (0.62, 0.97)	0.70 (0.46, 0.88)	0.89 (0.67, 0.99)
mean (SD) rate of incidence	3.1 (3.4) <sup>1</sup>	3.1 (3.4)	6.6 (7.6)	9.7 (10.2)	12.1 (18.8)
median rate of incidence	1.8 <sup>1</sup>	1.8	4.2	5.4	5.8

<sup>1</sup> Patient ENB-010-10-16-11 excluded due to treatment duration of less than one week.

Rate of incidence calculated by number of events/duration of treatment in years;  
95% CI for proportion of affected patients determined by Clopper-Pearson method  
70/102 (69%) patients had an ISR adverse event, of which 58 patients were ADA+

**Table 22. Incidence of IAR by quartiles of % inhibition of FcD10**

(Source: Table 5 of Applicant's response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
FcD10 % Range	NA	[0 – 9.09]	[9.09 – 19.4]	[19.4 – 38.7]	[38.7 – 96.6]
patients with IAR in quartile / total patients in quartile	5 / 24	1 / 19	3 / 20	3 / 20	4 / 19
proportion of affected patients (95 % CI)	0.21 (0.07, 0.42)	0.05 (0.00, 0.26)	0.15 (0.03, 0.38)	0.15 (0.03, 0.38)	0.21 (0.06, 0.46)
mean (SD) rate of incidence	4.6 (5.2) <sup>1</sup>	2.0 <sup>2</sup>	2.0 (1.3)	1 (1.4)	0.6 (0.2)
median rate of incidence	2.9 <sup>1</sup>	2.0 <sup>2</sup>	1.4	0.2	0.6

<sup>1</sup> Patient ENB-002-08-03-01 was excluded from rate of incidence calculations due to only one dose administered.

<sup>2</sup> Reported value is not mean or median as N=1.

Rate of incidence calculated by number of events/duration of treatment in years;  
95% CI for proportion of affected patients determined by Clopper-Pearson method;  
16/102 (16%) patients had an IAR adverse event, of which 11 patients were ADA+

**Table 23. Incidence of ectopic calcification by quartiles of % inhibition of FcD10**  
(Source: Table 6 of Applicant’s response to FDA IR dated July 7, 2015)

Statistic	ADA-	ADA+			
		Quartile 1	Quartile 2	Quartile 3	Quartile 4
FcD10 % Range	NA	[0 – 9.09]	[9.09 – 19.4]	[19.4 – 38.7]	[38.7 – 96.6]
patients with Ectopic Calcification in quartile / total patients in quartile	4 / 24	6 / 19	6 / 20	4 / 20	5 / 19
proportion of affected patients (95 % CI)	0.17 (0.05, 0.37)	0.32 (0.13, 0.57)	0.30 (0.12, 0.54)	0.20 (0.06, 0.44)	0.26 (0.09, 0.51)
mean (SD) rate of incidence	1.3 (0.7)	0.4 (0.2)	0.2 (0)	0.2 (0.1)	0.3 (0.1)
median rate of incidence	1.3	0.4	0.2	0.2	0.3

Rate of incidence calculated by number of events/duration of treatment in years  
95% CI for proportion of affected patients determined by Clopper-Pearson method  
25/102 (25%) patients had an Ectopic Calcification adverse event, of which 21 patients were ADA+

## 2.7 Extrinsic Factors

### 2.7.1 What extrinsic factors influence exposure and/or response, and what is the impact of any differences in exposure on effectiveness or safety responses?

No formal studies on extrinsic factors have been conducted.

## 2.8 General Biopharmaceutics

In the Pop-PK analysis, three product characteristics have been identified to influence the PK characteristics of asfotase alfa in patients with HPP, including (b) (4) formulation strength, manufacturing batch size, as graphically illustrated in Figure 8. The effects of these product quality/characteristics are further evaluated and discussed below.

### 2.8.1 Was the manufacturing process changed during the development program? (Include a table listing all the products used throughout the clinical development programs.) What formulation strength is available for the to-be-marketed drug product?

Throughout the development of asfotase alfa, the manufacturing process development has been shared by (b) (4) and Alexion. Initial drug substance batches intended for clinical studies were manufactured by (b) (4) manufactured at a (b) (4) cell culture scale. The manufacturing process was (b) (4) to a (b) (4) cell culture process and transferred to (b) (4) to meet expected demand. Batches used for clinical studies are summarized in Table 24.

Two formulation strengths were used to accommodate a wide range of doses administered during the development program. Drug product was supplied as a sterile aqueous solution for SC administration containing asfotase alfa at a concentration of 40 mg/mL or 100 mg/mL in (b) (4) sodium phosphate, (b) (4) sodium chloride in a 2 mL glass vial.

**Table 24. Use of asfotase alfa drug product lots from different scales in clinical development program**

(Source: Table 4 of Module 2.7.4 Summary of Biopharmaceutic Studies)

Asfotase alfa Drug Product Manufacturing Scale	Clinical Studies
(b) (4)	ENB-001-08, ENB-002-08/ENB-003-08 <sup>a</sup> , ENB-006-09/ENB-008-10 <sup>a</sup> , ENB-009-10 <sup>b</sup> and ENB-010-10 <sup>b</sup>
(b) (4)	ENB-002-08/ENB-003-08 <sup>a</sup> , ENB-006-09/ENB-008-10 <sup>a</sup> , ENB-009-10 <sup>b</sup> and ENB-010-10 <sup>b</sup>

<sup>a</sup> Ongoing extension study.

<sup>b</sup> Ongoing study.

**2.8.2 Was the proposed to-be-marketed formulation comparable to the formulation used in the pivotal clinical trials with respect to PK?**

Generally speaking, the to-be-marketed formulations have been evaluated in the clinical trials because the characteristics of products used in clinical trials encompassed the characteristics of the to-be-marketed product. For instance, the clinical trial materials included (b) (4) and (b) (4) scales and the (b) (4) scale is the to-be-marketed product. Similarly, the 40 mg/mL and 100 mg/mL formulations were used in clinical trials and also are proposed formulation strengths for the to-be-marketed product. With regards to the (b) (4) the clinical trial materials ranged from (b) (4) whereas the proposed specification for the to-be-marketed product is (b) (4).

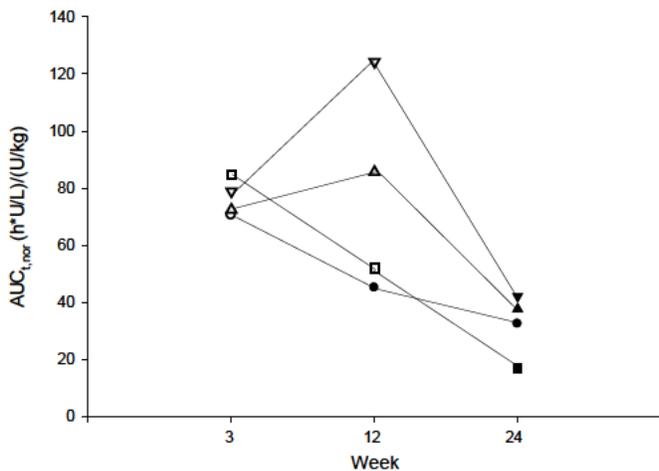
However, the clinical experience with the (b) (4) product is more limited compared to that of the (b) (4) material because it was introduced later in development. Therefore, analyses were conducted to evaluate the potential difference in PK between (b) (4) and (b) (4) product scale.

In the absence of a stand-alone PK comparability study for the (b) (4) and (b) (4) scale products, the Clinical Pharmacology reviewer performed an intra-subject comparison of the PK data associated with the (b) (4) scale and the (b) (4) scale product in four subjects who received both products at three study visits in Study ENB-009-10. These subjects initially received the (b) (4) scale product and transitioned to the (b) (4) scale product. The comparison was based on dose normalized AUC<sub>t</sub> over the dosing interval (AUC<sub>t,nor</sub>) from NCA at Weeks 3, 12, and 24, where the calculation of AUC<sub>t,nor</sub> is described in Section 2.5.3. The individual age of the subjects was 14, 15, 55, and 57 years old and the median weight was 64.6 (56.6 – 78.5) kg. All PK concentrations were analyzed (b) (4) method (see Section 2.9.2).

As shown in Figure 13, the AUC<sub>t,nor</sub> of the (b) (4) scale product was approximately 2-fold lower than that of the (b) (4) scale product. Further investigation revealed that the (b) (4) product had a (b) (4)

(b) (4) Of note, similar degree of reduction in exposure by C<sub>avg,ss</sub> is predicted by the Pop-PK model (b) (4)

**Figure 13.  $AUC_{f,nor}$  at Weeks 3, 12, and 24 for four subjects in ENB-009-10. Open symbols represent (b) (4) scale product and closed symbols represent (b) (4) scale product. Each patient is represented by a different symbol in the figure.**



Of note, the observed data for the (b) (4) scale product used in the clinical trial may not be reflective of the final to-be-marketed product. As per CMC review team, (b) (4) was only observed in the specific product lot of (b) (4) scale product produced during the initial period when the (b) (4) scale product was (b) (4) to the (b) (4) scale product. The manufacturing process improved over time, and the recent product lots were produced with better consistency (b) (4). The (b) (4) value of the current product lots ranges from (b) (4). Therefore, the final-to-be-marketed product is expected to have a higher exposure (b) (4).

Overall, our analysis indicates that (b) (4) is not acceptable for future to-be-marketed product (b) (4). Furthermore, the (b) (4) is a highly important product quality attribute as it has major influence on the systemic exposure; therefore, the product specification for (b) (4) should be thoughtfully defined to facilitate achieving therapeutic effects.

Using a Pop-PK approach, the Applicant also estimated the comparative bioavailability for the drug product manufactured at (b) (4) scale, with the drug product manufactured at (b) (4) scale as the reference. The (b) (4) scale product was selected as the reference because it represented 86.5% of the PK data from the (b) (4) product lots.

The estimated comparative bioavailability was (b) (4) with 90% confidence interval (CI) of (b) (4) or the drug product manufactured at (b) (4) scale with the (b) (4) scale product as the reference. Since the 90% CI on the point estimate of comparative bioavailability was contained within (b) (4) goal range, the Applicant claimed that the to-be-marketed drug product at the (b) (4) scale is expected to have similar safety and efficacy to the drug product at the (b) (4) scale.

**2.8.3 What is the impact of (b) (4) on asfotase alfa PK? Should the (b) (4) specifications be restricted based on what is known about the E-R relationships in the target population and their variability?**

Results of Pop-PK analysis demonstrated significant impact of (b) (4) on asfotase alfa exposure. There was an approximate (b) (4) difference in the  $C_{avg,ss}$  (Figure 8) of asfotase alfa between the extremes of proposed (b) (4), and the low end of the range has the potential to have concentrations below the range for plateau of maximal response for the study endpoints mentioned in Section 2.4.1. Raising the lower limit (b) (4) (b) (4) would provide more consistent exposure to the patient at the needed concentrations of asfotase alfa.

As illustrated in Figure 8, a (b) (4) which was associated with a near-maximum response in the E-R curves (Figure 3, Figure 4, and Figure 5). In addition, there was clinical experience with product lots with (b) (4) in asfotase alfa clinical trials, and clinical efficacy was demonstrated in juvenile-onset HPP. Therefore, it would be desirable to (b) (4) the (b) (4) of the (b) (4) specification to at least (b) (4) from the proposed (b) (4) for future product lots of the to-be-marketed product.

On the other hand, it may be prudent to maintain the (b) (4) of the (b) (4) specification (b) (4) as proposed by the Applicant. In asfotase alfa clinical trials, incidence of ectopic calcification where the deposit was found in the cornea and kidney were observed and considered adverse events. While the long-term sequelae of these adverse events remained to be assessed, it is unknown whether targeting higher asfotase alfa concentration would have any impact on the long-term safety of asfotase alfa. Therefore, (b) (4)

**2.8.4 How often were product lots with (b) (4) used in asfotase alfa clinical trials? How did this usage impact the efficacy evaluation of asfotase alfa in HPP clinical trials as well as the recommendation for the (b) (4) specification?**

Among the product lots used during the clinical development of asfotase alfa, the (b) (4) (b) (4) In the infantile-onset HPP patients, the (b) (4) product was used in <10% of the time (Table 25) during Studies ENB-002-08/ENB-003-08 and ENB-010-10; therefore, the impact of this (b) (4) product on efficacy evaluation should be minimal. Given the lack of data with the (b) (4) product, the overall clinical experience supports the use of products with (b) (4) in infantile-onset HPP. In juvenile-onset HPP patients (Study ENB-006-09/ENB-008-10), products with (b) (4) of (b) (4) and (b) (4) were used for a total of approximately (b) (4) % of the time during the study. The use of the (b) (4) product is likely to be acceptable in juvenile-onset HPP, due to the acceptable efficacy for asfotase alfa shown in this patient population.

Overall, future asfotase alfa product lots with (b) (4) are not recommended for clinical use in patients with either the infantile- or juvenile-onset HPP because

(b) (4) (see Section 2.8.2).

To address the question of whether the product lots with (b) (4) might have affected the efficacy of asfotase alfa in HPP patients, an IR dated March 10, 2015 was sent to the Applicant requesting additional information on the use of these products in clinical trials. The asfotase alfa products used across all the studies in the clinical development program had (b) (4) ranging from (b) (4)

Table 25 summarizes the use of the product lots with different (b) (4) values in asfotase alfa clinical trials.

For the infantile-onset HPP, all of the patients in ENB-002-08/ENB-003-08 and ENB-010-10 received products with (b) (4) ranging from (b) (4). The (b) (4) was used in 80% and 25% of the subjects in ENB-002-008/ENB-003-08 and ENB010-10, respectively. No infantile-onset HPP patients received the (b) (4) product during these two clinical trials. With respect to the fraction of time when the product was used over the entire treatment period, the (b) (4) were used in > 92% of the time, whereas the (b) (4) product was used in < 10% of the time.

Since the (b) (4) product was used in <10% of the time during the clinical trials, the impact of this (b) (4) product on efficacy evaluation should be minimal. Given the lack of data with the (b) (4) product, the overall clinical experience supports the use of products with (b) (4) in infantile-onset HPP.

For the juvenile-onset HPP, most of the patients received products with (b) (4) ranging from (b) (4) throughout the treatment period. Approximately 40% of the time during the treatment duration these patients received products with (b) (4) products used in 13% and 28% of the time, respectively, in ENB-006-09. Because improvements in 6MWT and other PD endpoints were observed in the juvenile-onset HPP, the use of the (b) (4) product is likely to be acceptable due to its association with acceptable efficacy. The product with (b) (4) is not acceptable (b) (4) (see Section 2.8.2).

Study ENB-009-10 evaluated two dosing regimens that are lower than the proposed dosing regimen of 6 mg/kg/week. Hence, the usage of different product lots is irrelevant to the efficacy evaluation of asfotase afla under the currently proposed dosing regimen.

**Table 25. Usage of product lot with different (b) (4) values in asfotase alfa clinical trials**

	ENB-002-08/ ENB-003-08	ENB-010-10	ENB-006-09/ ENB-008-10	ENB-009-10
Patient Population	< 3 y.o. Infantile	≤ 5 y.o. Infantile	5 – 12 y.o. Infantile, juvenile	> 12 y.o. Infantile, juvenile, adult
Weeks on Treatment	222 ± 105 258 (0 – 312)	65.6 ± 61.8 47 (0 – 213)	221 ± 65.9 234 (4 – 260)	175 ± 32.8 192 (96 – 216)

Mean ± S.D; median (range)

**2.8.5 Was the 40 mg/mL comparable to the 100 mg/mL used in the pivotal clinical trials with respect to PK?**

The 100 mg/mL formulation strength product had lower bioavailability compared to the 40 mg/mL formulation strength product, but the impact on PK exposure is likely less than that of (b) (4). The clinical efficacy in infantile-onset HPP patients was demonstrated using 40 mg/mL in Studies ENB 010-10 and ENB 002-08/003-08, but clinical efficacy in these patients has not been demonstrated with the 100 mg/mL formulation strength. Given that the 100 mg/mL formulation has a lower exposure than the 40 mg/mL formulation strength, we recommend that only the 40 mg/mL formulation strength be used in the pediatric infantile-onset patients.

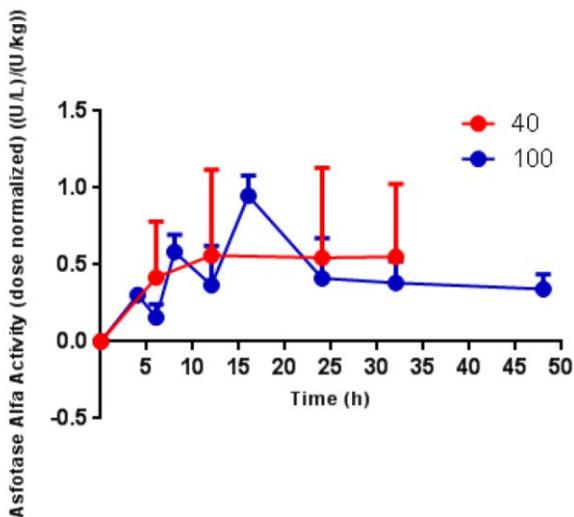
A stand-alone PK study was not conducted to determine the PK comparability between the 40 mg/mL and the 100 mg/mL formulation strength products. Instead, The Applicant performed the following analyses to assess the PK comparability using the PK data from the clinical studies.

Using the concentration-time profile following the first SC dose administration of asfotase alfa in 35 HPP patients (Figure 14), the Applicant compared the  $C_{max,nor}$  and  $AUC_{t,nor}$  obtained from the NCA method between the two formulation strengths. Of note, the subjects in the 40 mg/mL group were children ≤ 5 years old enrolled in Study ENB-010-10, whereas those in the 100 mg/mL group were > 5 years old enrolled in Studies ENB-006-09 and ENB-009-10.

The least-squares mean ratios for  $C_{max,nor}$  and  $AUC_{t,nor}$  for the 100 mg/mL formulation strength were 89.9 % (90% CI: (b) (4)) and 116 % (90% CI: (b) (4)), respectively, with the 40 mg/mL formulation strength as the reference product (Table 26).

In addition to the difference in patient characteristics between the two formulation strength groups as described above, the variability in the (b) (4) in the product lots is a confounding factor that may also apply to this NCA analysis comparing the two formulation strength products.

**Figure 14. Mean (SD) dose normalized asfotase alfa activity vs. time profiles following SC administration of the 40 mg/mL and 100 mg/mL formulation strength products at Week 1**  
(Source: Figure 2 of the Applicant’s response to IR dated January 26, 2015)



**Table 26. Comparison of asfotase alfa exposure following administration of 40 mg/mL (n = 19) and 100 mg/mL (n = 16) formation strength products**  
(Source: Table 13 of the Applicant’s response to IR dated January 26, 2015)

PK Parameters	Mean Ratio % (100 mg/mL) / 40 (mg/mL) <sup>a</sup>	LSM (40 mg/mL)	LSM (100 mg/mL)	Confidence Interval 90 Lower	Confidence Interval 90 Upper
$C_{max, nor}$	89.9	-0.7	-0.8	64.4	126.0
$AUC_{t, nor}$	116	2.4	2.6	84.9	158.0

<sup>a</sup> Antilogarithm of the difference (test minus reference) of the least squares means for logarithms.

Abbreviations: LSM = least-squares matching;  $C_{max, nor}$  = dose normalized maximum serum concentration post- dose;  $AUC_{t, nor}$  = dose normalized area under serum concentration-time curve to last measurable concentration.

The Applicant also analyzed the comparability of the two formulation strength product within the framework of Pop-PK analysis. The formulation strength factor was implemented as a covariate effect modulating both rate (absorption rate constant) and extent (relative bioavailability) of drug in the Pop-PK model, using the 40 mg/mL strength as the reference formulation strength.

After taking into consideration the differences in (b) (4) the estimate (90% CI) for the relative ratio of 100 mg/mL to 40 mg/mL formulation strength for relative bioavailability was (b) (4) indicating an approximate (b) (4) lower bioavailability for the 100 mg/L formulation strength compared to the 40 mg/mL formulation strength.

The Applicant performed a simulation of PK exposures between two formation strength products administered to patients with different body weights (Table 27). The ratio of AUC between a 20 kg weight child receiving 40 mg/mL strength (6 mg/kg weekly dose) and a 70 kg weight adult receiving 100 mg/mL strength (6 mg/kg weekly dose) is 0.94, very close to 1. It is because patients with smaller body size and weight will receive 40 mg/mL formulation strength and patients with larger body size and weight will receive 100 mg/mL formulation strength, and the difference in PK exposure due to formulation strength across the patient population would be minimized by the independent allometric impact on asfotase alfa PK. The Applicant's analyses supported that 100 mg/mL formulation achieves a lower systemic exposure of asfotase alfa compared to the 40 mg/mL formulation. Therefore, the potential for the formulation to impact the clinical outcome cannot be ruled out because of the correlation between exposure and response as described in Section 2.4.1.

**Table 27. Comparison of PK exposures between two formation strength products administered to patients with different body weights**

(Source: Table 3 the Applicant's response to IR dated January 26, 2015)

Strength (mg/mL)	Weight (kg)	$F_{SC}^{(4)}$	$F_{strength}$	CL/F1 (L/day)	Dose (mg)	AUC (mg*day/L)	AUC Ratio*
40	20	0.62		8.18	120	14.67	
100	70	0.62	0.762	26.88	420	15.62	0.94

\* The AUC ratio corresponds to 100 mg/mL strength over 40 mg/mL strength

### **2.8.6 What is the impact of formulation strength on immunogenicity related findings?**

Uncertainty remains about the impact of formulation strength on immunogenicity related findings, which include ISR, IAR, and immunogenicity rate, due to the small number of subjects in the clinical trials of the asfotase alfa development program and the difference in patient characteristics receiving the 40 mg/mL and the 100 mg/mL formulation strength products.

The immunogenicity related finding between the 40 mg/mL and the 100 mg/mL formulation strength products are summarized in two groups of subjects who exclusively received only one of the formulation strength products (Table 28). All patients in the 40 mg/mL group were below 6 years of age at the time of first dose, whereas patients in the 100 mg/mL group were above 10 years of age at first dose. Additionally, majority of the patients in the 40 mg/mL group received asfotase alfa 3 times per week compared to 6 times per week in the 100 mg/mL group.

ISR were reported in a higher proportion of patients in the 100 mg/mL group (85.7%) compared to the 40 mg/mL group (55.9%). This observation is likely due to the inherently greater opportunity for the occurrence of an ISR because of a higher dosing frequency (i.e., six times per week) in the 100 mg/mL group.

The incidences of IAR and ADA positivity appear to be similar between the two formulation strengths.

**Table 28. Summary of immunogenicity related findings between the 40 mg/mL and 100 mg/mL formulation strength products in different subjects exclusively received only one of the formulation strength products**

	40 mg/mL	100 mg/mL
N	34	7
Age		
Mean (SD)	1.99 (2.00)	39.0 (25.3)
Median (Range)	1.08 (0 – 6.0)	44.7 (10.1 – 66.8)
Dosing		
3x Weekly	32 (94.1%)	1 (14.3%)
6x Weekly	1 (2.9%)	6 (85.7%)
Adverse Event		
Injection site reactions	19 (55.9%)	6 (85.7%)
Injection/infusion associated reactions	8 (23.5%)	1 (14.3%)
Serious adverse events		
Injection/infusion associated reactions	1 (0.03%)	0 (0%)
ADA Status		
n	33	6
ADA-	9 (27.3%)	1 (16.7%)
ADA+	24 (72.7%)	5 (83.3%)

## 2.9 Analytical Section

### 2.9.1 What bioanalytical methods are used to assess the therapeutic protein concentrations, PD markers, and immunogenicity?

A number of assays were used in the clinical development program of asfotase alfa to determine the asfotase alfa concentrations (in terms of enzyme activity), PPi and PLP levels, ADA, and NAb. These assay methods as well as the validation reports submitted are summarized in Table 29.

### 2.9.2 Briefly describe the methods that are used to assess asfotase alfa concentration and summarize the assay performance. Please provide tables for each assay to address the below questions

Asfotase alfa concentration data were generated by two analytical laboratories, (b) (4) (b) (4). Initial assay development and sample analysis was conducted at (b) (4) (from May 2008 to October 2009). The method was later transferred to (b) (4) (March 2010), where the assay was further refined and re-validated. Table 30 presents the number of samples in each study that were analyzed by each of two methods. The two methods were similar in methodology and procedures.

A retrospective cross-validation of the two methods was conducted, and the bioanalytical method was explored as a covariate in the Pop-PK analysis. PK data generated using both methods were

used directly without transformation for Pop-PK analysis and pooled PK-PD analysis for asfotase alfa.

**Table 29. Summary of bioanalytical assay methods used in clinical studies**

Analyte	Assessment Type	Method 1 Lab	Method 2 Lab	Method 3 Lab	Validation Reports Submitted
Asfotase alfa	PK	(b) (4)	(b) (4)	(b) (4)	(b) (4)-001059 (4)
PPi	PD				Amendment 3 (b) (4) MBR10-119 (b) (4)
PLP	PD				315267 (b) (4)
ADA	Immunogenicity				Validation summary (b) (4) VAL310.R4 (b) (4)
NAb	Immunogenicity				VAL-2008-502VR (b) (4) KGV2 and KGV4 (b) (4) MYY2 (b) (4)

PPi, inorganic pyrophosphate; PLP, pyridoxal-5'-phosphate; ADA, antidrug antibodies; NAb, neutralizing antibodies; PK, pharmacokinetic; PD, pharmacodynamics;

<sup>1</sup>Based on the method of (b) (4) without formal validation

<sup>2</sup>(b) (4) Research was formerly known as (b) (4)

<sup>3</sup>PLP analysis was outsourced to (b) (4) through (b) (4)

**Table 30. Summary of PK data obtained from the (b) (4) and (b) (4) methods**  
(Source: Table 8 of Module 2.7.1 Summary of Biopharmaceutic Studies)

Clinical Study	(b) (4) Method	(b) (4) Method	Combined
ENB-001-08	206	0	206
ENB-002-08/ENB-003-08	151	120	271
ENB-006-09/ENB-008-10	0	283	283
ENB-009-10	0	375	375
ENB-010-10	0	387	387
<b>Total Number</b>	<b>357</b>	<b>1165</b>	<b>1522</b>
<b>Percentage</b>	<b>23.5%</b>	<b>76.5%</b>	<b>100%</b>

### 2.9.2.1 PK Assay Description

The assay for asfotase alfa is based upon the catalytic activity of the TNSALP domain of the molecule under steady state conditions. Para-nitrophenyl phosphate (pNPP) is the alkaline phosphatase substrate used in the assay, which is broken down to para-nitrophenol (pNP). The reaction velocity for the given substrate, at steady state, is described by the Michaelis-Menten equation:

$$v = \frac{V_{\max} [S]}{K_M + [S]}$$

Calibration curves were established in terms of protein concentration (ng/mL) and the results converted to activity units (U/L). For the standard curve for the (b) (4) and the (b) (4) assay the conversion formula for protein concentration (ng/mL) to activity units (U/L) is given by:

(b) (4)

One activity unit (U) corresponds to the rate of formation of 1  $\mu\text{mol}/\text{min}$  of pNP from pNPP. The substrate pNPP was used at a saturating concentration of 1mM. The reaction proceeded at the  $V_{\text{max}}$  rate within the observation window. The product, pNP, is a yellow colored compound, with a maximal absorption at 405 nm and used for quantification using colorimetric plate reader.

#### 2.9.2.2 Assay by (b) (4)

(b) (4) has validated 2 versions of the asfotase alfa activity assay. Version -1 documented in SOP CT-00021 DRAFT and Rev.2 (Revision 2) did not use plate calibrators ((b) (4) study (b) (4)-2008-498VR and (b) (4) study CB-001059 Amendment 1). Version -2 of the assay, documented in SOP CT-00021 Rev.3, SOP CT-00021 Rev.4 and SOP CT-00021 Rev.5, added a calibration curve to improve the robustness of the assay ((b) (4) study (b) (4)-001059 Amendment 3). For sample analysis, SOP CT-00021 Rev.4 and Rev.5 were followed.

Table 31 and Table 32 summarize the validation results of (b) (4) Method Version -1 and Version -2, respectively.

#### **Reviewer's Comments**

*The performance of the validated (b) (4) method did not conform to the recommendations in the September 2013 FDA Guidance for Industry. Bioanalytical Method Validation. Specifically, the inter-assay accuracy for QCI was 74% with >20% relative error (Table 31) and incurred sample reanalysis was not preformed. Nonetheless, because 23.5 % (Table 29) of the PK data were obtained from the (b) (4) method and the use of the PK data from the (b) (4) method had little impact on Pop-PK parameter estimates (see Section 2.9.2.5 below), it is acceptable to include the PK data generated by the (b) (4) method in the Pop-PK model.*

**Table 31. Summary of validation results of (b) (4) Method Version -1**  
 (Source: Table 9 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Assay Parameters	Results Summary				
Method	(b) (4)				
Analyte	Asfotase alfa activity				
Matrix	Serum				
Reportable Range	(b) (4)				
Sensitivity (LLOQ)	(b) (4)				
QC1, QC2 and QC3	(b) (4)				
QC Intra-Assay Statistics in Serum	Level	Activity (mAbs/min)	Concentration (ng/mL)	Precision (% CV)	Accuracy (% Recovery)
	QC1	(b) (4)			
	QC2	(b) (4)			
	QC3	(b) (4)			
QC Inter-Assay Statistics in Serum	Level	Activity (mAbs/min)	Concentration (ng/mL)	Precision (% CV)	Accuracy (% Recovery)
	QC1	(b) (4)			
	QC2	(b) (4)			
	QC3	(b) (4)			
QC Inter-Assay Statistics in Assay Diluent	Level	Activity (mAbs/min)	Concentration (ng/mL)	Precision (% CV)	Accuracy <sup>b</sup> (% Recovery)
	QC1	(b) (4)			
	QC2	(b) (4)			
	QC3	(b) (4)			
Stability at (b) (4)	(b) (4)				
Assay Parameters	Results Summary				
Freeze/Thaw Stability	(b) (4)				
Frozen Matrix Storage Stability	(b) (4)				
Dilutional Linearity	(b) (4)				

(b) (4) was not presented in the validation report (VAL-2008-498VR); the data was calculated from the data presented in the validation report.

**Table 32. Summary of validation results of (b) (4) Method Version -2 (b) (4) study CB-001059 Amendment 3)**

(Source: Table 11 of Module 2.7.1 Summary of Biopharmaceutic Studies)

Assay Parameters		Results Summary			
Method	(b) (4)				
Analyte	Asfotase alfa activity				
Matrix	Human serum				
Reportable Range	(b) (4)				
Curve fitting	(b) (4)				
Sensitivity (LLOQ)	(b) (4)				
QC1, QC2 and QC3	(b) (4)				
QC Inter-Assay Statistics in Human Serum (%)	Level	Activity (U/L)	Concentration (ng/mL)	Precision (% CV)	Accuracy % Recovery
	QC1	(b) (4)			
	QC2	(b) (4)			
	QC3	(b) (4)			
QC Inter-Assay Statistics in Assay Diluent (%)	Level	Activity (U/L)	Concentration (ng/mL)	Precision (% CV)	Accuracy % Recovery
	QC1	(b) (4)			
	QC2	(b) (4)			
	QC3	(b) (4)			
Dilution Linearity	(b) (4)				

**2.9.2.3 Assay (b) (4)**

Table 33 summarizes the validation results of the (b) (4) method.

**Reviewer’s Comments**

*The performance of the (b) (4) method is acceptable.*

**Table 33. Summary of Validation Results of the (b) (4) Method (MBR10-119)**  
 (Source: Table 15 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Parameter	Results Summary			
Method	(b) (4) PBA10-119.8)			
Analyte	Asfotase alfa activity			
Matrix	Serum			
Curve fitting	Linear, non weighted			
Standard Curve Range	(b) (4)			
Effective Curve Range Considering Assay MRD				
Sensitivity (LLOQ)				
QC Intra-Assay Statistics	Level	Concentration (ng/mL)	Precision (% CV)	Accuracy (as %RE)
	LLOQ	(b) (4)		
	Low	(b) (4)		
	Mid			
	High			
QC Inter-Assay Statistics	Low	(b) (4)		
	Mid			
	High			
Thawed Matrix Stability	(b) (4)			
Freeze/Thaw Stability	(b) (4)			
Frozen Matrix Storage Stability				
Working Stock Stability (mg/mL)	(b) (4)			
Dilutional Linearity	(b) (4)			

Parameter	Results Summary
Selectivity	All 10/10 lots tested < LLOQ (b) (4)
Specificity	All 10/10 lots (b) (4) of asfotase were acceptable (b) (4)
Hemolysis	(b) (4)
Lipemia	All degrees of lipemia (low, medium and high) were acceptable
Incurred Sample Re-analysis (Section 1.3.11.1)	(b) (4) % of the samples accepted for all three clinical studies below ENB-002-08, (b) (4) study number MBR10-135 ENB-006-09, (b) (4) study number MBR10-137 ENB-009-10, (b) (4) study number MBR10-326

**2.9.2.4 Retrospective Cross-Validation of (b) (4) and (b) (4) Methods**

At the time of the asfotase alfa PK assay transfer from (b) (4) to (b) (4) Enobia did not conduct a formal comparability study of the methods from the two laboratories. A retrospective cross-validation of the analytical methods was requested by Alexion and conducted in October 2013 at (b) (4) and (b) (4) using their respective methods. The test samples selected for the cross-validation exercise included 51 incurred samples from asfotase alfa clinical studies that were covered under available sample stability data and 30 samples spiked with asfotase alfa in pooled healthy human volunteer serum at various concentrations.

The predefined acceptance criterion was percent difference from at least (b) (4) of the sample results must be  $\leq 30\%$ . The percent difference of the results was determined using the following equation:

$$\frac{(\text{b}) (4) - (\text{b}) (4)}{(\text{b}) (4)} \times 100$$

Table 34 summarizes the results of the cross-validation of the (b) (4) and (b) (4) method; 93% of the comparisons fell within the acceptance criteria. Based on these results, the Applicant claimed that the data from the (b) (4) and (b) (4) methods were comparable.

**Table 34. Summary of the cross-validation results of the (b) (4) and (b) (4) methods**  
(Source: Table 16 of Module 2.7.1 Summary of Biopharmaceutic Studies)

Samples	N (total assayed)	N (difference could not be calculated)	N (difference calculated) <sup>a</sup>	N (samples with $\leq 30\%$ difference)	% (samples within $\leq 30\%$ difference)	Comment
Incurred Samples	51	5	46	43	93	Pass
Spiked Controls	30	3	27	25	93	Pass
<b>Total</b>	<b>81</b>	<b>8</b>	<b>73</b>	<b>68</b>	<b>93</b>	<b>Pass</b>

<sup>a</sup> When results from (b) (4) or (b) (4) were  $< \text{LLOQ}$ , the % difference could not be calculated or compared because the LLOQ for the two methods was different. The LLOQ for the (b) (4) method = 120.6 U/L with a MRD of 1:10, whereas the LLOQ for the (b) (4) method = 12.1 U/L.

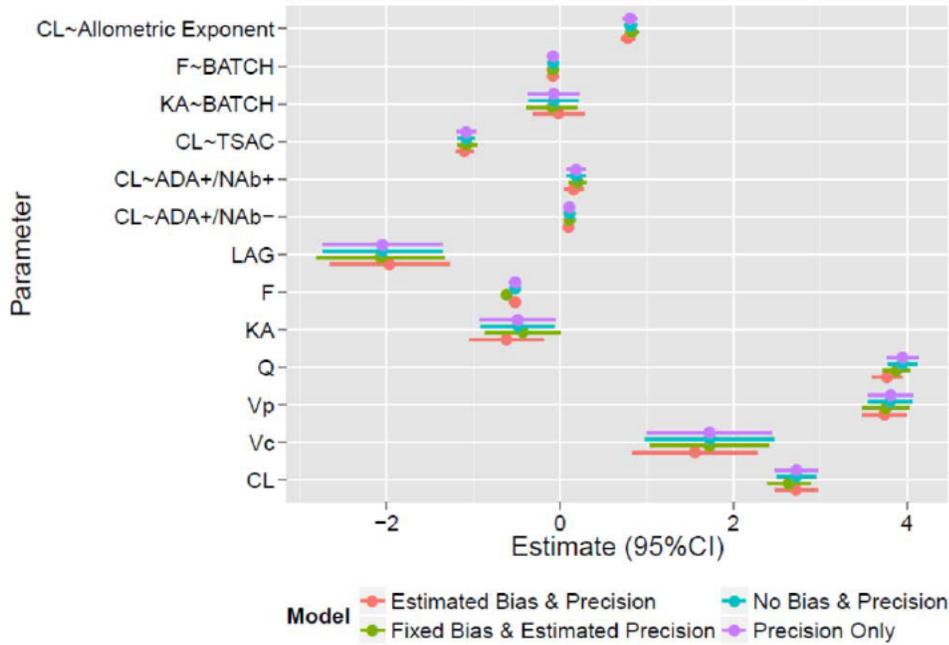
### **Reviewer's Comments**

*While the aggregate results of the cross-validation showed that 93% of the samples were within  $\leq 30\%$  difference between the two methods, examination of the individual data revealed that the concentrations from the (b) (4) method were consistently lower than those from the (b) (4) method. In other words, there was a bias towards lower concentrations analyzed the (b) (4) method. Nonetheless, the use of the (b) (4) data was shown to have little impact on Pop-PK parameter estimates by the Applicant (see Section 2.9.2.5 below). It is acceptable to include the PK data generated by the (b) (4) method in the Pop-PK model.*

#### **2.9.2.5 What is the impact of PK assay on asfotase alfa PK?**

In addition to the retrospective cross-validation of the (b) (4) and (b) (4) methods, the Applicant further assessed the impact of PK assay on estimated Pop-PK model parameters. Four different error models with different combinations of bias and precision information between the two methods were included in the Pop-PK model, and the resulting Pop-PK parameters from each of the four models were compared to determine if the Pop-PK parameters change. Results of the PK parameter estimate and confidence interval from these models showed minimum impact of assay in all cases (Figure 15), suggesting that the final Pop-PK model required no accommodation for assay differences.

**Figure 15. Evaluation of Inclusion of Assay-related Bias and Variance Parameters**  
 (Source: Figure 52 of Alexion’s Population PKPD report)



**2.9.3 Briefly describe the methods that are used to assess the PD marker PPI and summarize the assay performance.**

Enobia developed an assay for the determination of PPI in plasma, based on the method of (b) (4) without formal validation. The method was used for sample analysis between 2008 and 2011. In late 2010, the analytical method was transferred to (b) (4) where modifications were made to the assay prior to the validation.

**2.9.3.1 Assay Description**

The PPI method is based upon a (b) (4) (b) (4) illustrated in Figure 16 and is based on the method by (b) (4)

**Figure 16. PPI assay reaction scheme**

(Source: Figure 5 of Module 2.7.1 Summary of Biopharmaceutic Studies)



2.9.3.2 (b) (4) Assay

Table 35 summarizes the validation results of the (b) (4) method.

**Reviewer's Comments**

*The performance of the (b) (4) method is acceptable.*

**Table 35. Summary of the validation results of the (b) (4) method ( (b) (4) study 315267)**  
 (Source: Table 21 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Parameter	Results Summary			
Method	(b) (4) AP.315267.PPI.14			
Analyte	Inorganic pyrophosphate (PPi)			
Matrix	(b) (4)			
Curve fitting	(b) (4)			
Standard Curve Range	(b) (4)			
LLOQ-1 and -2	(b) (4)			
ULOQ	(b) (4)			
Low, Mid and High QC	(b) (4)			
QC Intra-Assay Statistics <sup>a</sup>	Level	Conc. (µM)	Precision (% CV)	Accuracy (% recovery)
	LLOQ-1	(b) (4)		
	LLOQ-2	(b) (4)		
	Low	(b) (4)		
	Mid	(b) (4)		
	High	(b) (4)		
	ULOQ	(b) (4)		
QC Inter-Assay Statistics	Level	Conc. (µM)	Precision (%CV)	Accuracy (% recovery)
	LLOQ-1	(b) (4)		
	LLOQ-2	(b) (4)		
	Low	(b) (4)		
	Mid	(b) (4)		
	High	(b) (4)		
	ULOQ	(b) (4)		
Thawed Matrix Stability	(b) (4)			
Freeze/Thaw Stability	(b) (4)			
Frozen Matrix Storage Stability <sup>d</sup>	(b) (4)			
Dilutional Linearity	(b) (4)			
Specificity	(b) (4)			
Hemolysis	(b) (4)			
Lipemia	All degrees of lipemia were acceptable in 2 of 3 donors			

<sup>a</sup> Intra-assay descriptive ranges represent values observed over 6 assays

<sup>b</sup> One assay had a high variability %CV=(b) (4)%, exclusion of this value results in a range of (b) (4)%.

<sup>c</sup> 5 out of 6 intra-assay runs met the acceptance criteria (b) (4)%.

<sup>d</sup> Additional stability assessments are described in Section 1.4.6.

<sup>e</sup> Gradations of hemolysis evaluated ranges from none (N) to highest H+++ . Graded hemolysed samples were provided by (b) (4)

### 2.9.3.3 Comparability Study of the Enobia and the (b) (4) PPi Methods

The comparability study of the method-1 employed at Enobia and the method-2 validated at (b) (4) were performed from January 2011 to June 2011. Results for the comparability study demonstrated that both assays yielded comparable results Table 35. The percent difference for 95.8% of the samples (23 out 24 samples) was within ± 25% (Table 36).

**Table 36. Summary of results from the comparability study comparing the Enobia and (b) (4) PPI methods**

(Source: Table 23 of Module 2.7.1 Summary of Biopharmaceutic Studies)

Site(s)	Site of Analysis and Method	Number of Samples Tested <sup>a</sup>	% of Samples with a %Difference ≤30% Between Methods
Enobia	Enobia - Methods 1 and 2	8 <sup>b</sup>	100%
Enobia & (b) (4)	Enobia – Method 1	13 <sup>c,d</sup>	92.3%
	(b) (4) Method 2		
Enobia & (b) (4)	Enobia – Method 2	3 <sup>e</sup>	100%
	(b) (4) Method 2		
Overall comparison results		24	95.8%

<sup>a</sup> All plasma samples evaluated were collected using SC3.

<sup>b</sup> 3 separate pools of plasma and 5 QC samples spiked with PPI in one plasma pool.

<sup>c</sup> 10 lots of plasma collected using SC3 from individual donors.

<sup>d</sup> 7 plasma samples collected under studies ENB-003-08 and ENB-010-10. Concentration < LLOQ was reported in 4 samples at either or both (b) (4) and Enobia. The % Difference was not calculated for these 4 samples.

<sup>e</sup> PPI QC samples prepared at three concentration levels in human plasma in SC3 at (b) (4) and sent blinded to Enobia. These samples were tested in both labs.

**2.9.4 What are the sample collection methods for the PD marker PPI and what data are included in data analysis?**

The two PPI methods (Enobia and (b) (4) and three different sample collection methods (SC1, SC2, and SC3 described below) were used during clinical Studies ENB-001-08, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10 and ENB-010-10. All samples in Study ENB-009-10 were collected using SC3 and analyzed by the PPI method by (b) (4) (see Section 2.9.4).

- SC1: (b) (4)
- SC2: (b) (4)
- SC3: (b) (4)

Only PPI data from samples collected using SC3 were used for the pooled PK-PD efficacy analyses (Table 37) because the Enobia and (b) (4) methods were comparable using samples collected using SC3 (see Section 2.9.3.3). In total, 86% of the PPI data were included for pooled PK-PD analysis, with 14% being excluded.

**Table 37. PPI data across clinical studies by collection methods and disposition of the data for pooled PK-PD analysis**

(Source: Table 19 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Clinical Study	Assayed by Enobia			Assayed by (b) (4)	Total Records	Samples Available for PK-PD Analysis n (%) <sup>a</sup>
	SC1	SC2 (b) (4)	SC3	SC3 (b) (4)		
ENB-001-08	27	22	0	0	49	(b) (4)
ENB-002-08/ ENB-003-08	2	34	85	36	157	(b) (4)
ENB-006-09/ ENB-008-10	0	0	96	52	148	(b) (4)
ENB-009-10	0	0	0	189	189	(b) (4)
ENB-010-10	0	0	18	196	214	(b) (4)
<b>Total Number</b>	<b>29</b>	<b>56</b>	<b>199</b>	<b>473</b>	<b>757</b>	(b) (4)
<b>Percentage</b>	<b>4</b>	<b>7</b>	<b>26</b>	<b>63</b>	<b>100%</b>	(b) (4)
Usage	Excluded <sup>b</sup>	Excluded <sup>c</sup>	Included	Included		(b) (4)

<sup>a</sup> As noted in the last row of the table, some samples were excluded for analytical reasons (inappropriate matrix).

Source: PK-PD report

**2.9.5 Briefly describe the methods that are used to assess the PD marker PLP and summarize the assay performance.**

PLP was initially measured by a (b) (4) developed by (b) (4) through (b) (4). This assay was contracted to (b) (4). Subsequently, the PLP liquid chromatography tandem mass spectroscopy assay (LC/MS/MS) was validated at (b) (4) to support clinical analysis of samples from ongoing studies.

**2.9.5.1 (b) (4) Assay Description**

The (b) (4) method involved a (b) (4)

**Figure 17. Reaction schema of the (b) (4) validated by (b) (4) for PLP measurement**  
 (Source: Figure 7 of Module 2.7.1 Summary of Biopharmaceutic Studies)



**2.9.5.2 (b) (4) Assay Description and Validation**

The (b) (4) method utilized (b) (4). The validation results of the assay are summarized in Table 38.

**Table 38. Summary of validation results for PLP assay at (b) (4)**  
 (Source: Table 28 of Module 2.7.1 Summary of Biopharmaceutic Studies)

Parameter	Results Summary			
Method	(b) (4) (BAM 310)			
Analyte	Pyridoxal-5'-phosphate			
Internal Standard	(b) (4)			
Matrix	(b) (4)			
Curve fitting	(b) (4)			
Standard Curve Range	(b) (4)			
Sensitivity (LLOQ)	(b) (4)			
ULOQ	(b) (4)			
QC1, QC2 and QC3	(b) (4)			
QC Intra-Assay Statistics	Level	Conc. (ng/mL)	Precision (%)	Accuracy (%)
	LLOQ	(b) (4)		
	Low	(b) (4)		
	Mid	(b) (4)		
	High	(b) (4)		
QC Inter-Assay Statistics	ULOQ	(b) (4)		
	LLOQ	(b) (4)		
	Low	(b) (4)		
	Mid	(b) (4)		
	High	(b) (4)		
ULOQ	(b) (4)			
In Process Stability	(b) (4)			
Processed Sample Stability	(b) (4)			
Autosampler (re-injection) stability	(b) (4)			
Freeze/Thaw Stability <sup>a</sup>	(b) (4)			

Parameter	Results Summary
Frozen Matrix Storage Stability <sup>a</sup>	(b) (4)
Dilutional Linearity	
Carryover <sup>b</sup>	
Selectivity	
Matrix Effect and Matrix Factor	
Hemolysis	No interference for all degrees of hemolysis (b) (4)
Lipemia	No interference for lipemia

<sup>a</sup> Additional stability assessments are described in Section 1.5.6

<sup>b</sup> Carry over results beyond (b) (4) are detailed in the text

### **Reviewer's Comments**

The performance of the (b) (4) is acceptable.

#### **2.9.5.3 Comparability of (b) (4), (b) (4) and (b) (4) LC/MS/MS Assay**

A direct comparison of the (b) (4), (b) (4) and (b) (4) assay was not performed because the (b) (4) method was retired in November 2010. Some samples from ENB-003-08, ENB-008-10, and ENB-009-10 were analyzed by both methods and used to establish a regression/correlation between the two methods ( $r^2 = 0.8581$ ). When only (b) (4), (b) (4) result was available, the result was transformed into the “(b) (4) LC/MS/MS equivalent” data using the following equation:

(b) (4)

#### ***2.9.6 What are the sample collection methods for the PD marker PLP and what data are included in data analysis?***

Samples for PLP were analyzed by 2 main laboratories ((b) (4) and multiple local labs. Both sets of data (b) (4) were used in pooled PK-PD efficacy analysis, whereas the data collected at local labs were excluded.

Table 39 summarizes the numbers of all PLP sample data included in the PK-PD. The majority (92%) of the PLP samples was available for PK/PD analysis, with 8% excluded due to local lab analysis or inappropriate sample matrix.

**Table 39. PLP data across clinical studies by collection methods and by local laboratories, and (b) (4)**  
 (Source: Table 25 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Clinical Study	Local Labs	Sample Results <sup>a</sup>				Total Samples	Total Samples Available for PK-PD analysis n (%) <sup>b</sup>
		K <sub>2</sub> EDTA (b) (4)	(b) (4)	(b) (4)	(b) (4)		
ENB-001-08	0	17	0	0	0	17	17 (100%)
ENB-002-08/ ENB-003-08	56	14	24	27	90	151	109 (72%)
ENB-006-09/ ENB-008-10	16	46	45	37	110	184	168 (91%)
ENB-009-10	0	19	0	0	189	208	208 (100%)
ENB-010-10	0	9	5	3	195	204	204 (100%)
<b>Total Number</b>	72	105	74	67	584	764	706 (92%)
<b>Usage</b>	Excluded	Included <sup>c</sup>	Excluded <sup>d</sup>	Excluded <sup>c</sup>	Included		

<sup>a</sup> Sample results includes all analytical results. In some instances a sample may have been analyzed using more than one method.

**2.9.7 Briefly describe the methods that are used to assess the immunogenicity and assay performance including sensitivity, specificity, precision, cut point, interference (including drug interference) and matrix, etc.**

Enobia implemented a risk-based approach for immunogenicity assessment in June 2010. The immunogenicity testing paradigm detects and characterizes the immunogenicity of asfotase alfa using a suite of validated assays as shown in Figure 18.

**Figure 18. Flow Chart for the Detection and Characterization of Treatment Emergent Anti-Drug Antibodies**

(Source: Figure 8 of Module 2.7.1 Summary of Biopharmaceutical Studies)



**2.9.7.1 What is binding ADA assay and its performance?**

The binding assay is based on an ADA bridge of immobilizing antigen (b) (4) and reporting antigen (b) (4). The bridge complex (Figure 19) is captured with (b) (4) asfotase alfa (b) (4)-asfotase). The measurement is based on a (b) (4)

(b) (4)

**Figure 19. Schematic diagram of the bridge complex formed with anti-asfotase alfa antibody**  
(Source: Figure 9 of Module 2.7.1 Summary of Biopharmaceutic Studies)

(b) (4)

All patient samples were subject to the screening assay. The screening assay cut point was established from a population of 50 drug-naïve normal donors analyzed in 3 separate runs. Using an upper 95<sup>th</sup> percentile of the statistical analysis, a fixed cut point as selected to give a 5% false positive rate.

Study samples that have screened potentially positive in the screening step were further analyzed in the confirmatory assay. This assay followed the same procedure (b) (4)

For each sample, the percent inhibition by the asfotase alfa assay inhibitor was calculated. The confirmatory cut-point was established from the statistical analysis of data from 50 drug naïve normal donors assayed with and without free asfotase alfa. Using an upper 99.9<sup>th</sup> percentile of the statistical analysis, the confirmatory assay cut point, equal to (b) (4) inhibition, was selected to give a 0.1% false positive rate.

Samples that confirmed positive were further evaluated in a (b) (4). For this assay (b) (4)

The characterization assay used the same format as described for the confirmatory assay. (b) (4)

(b) (4)

Table 40 summarizes the validation results for the ADA assay. According to the CMC reviewer, the performance of the binding ADA assay is acceptable.

**Table 40. Summary of validation results for ADA assay**  
(Source: Table 30 of Module 2.7.1 Summary of Biopharmaceutical Studies)

<b>Parameter</b>	<b>Result</b>		
<b>Method</b>	(b) (4) (ICDIM 86 Version 2.02)		
<b>Analyte</b>	Anti-asfotase alfa antibodies		
<b>Matrix</b>	Serum		
<b>Parameter</b>	<b>Result</b>		
<b>Sensitivity</b>	(b) (4)		
<b>PC</b> (b) (4)	(b) (4)		
<b>PC Conc.</b>	(b) (4)		
<b>Control Intra-Assay<sup>a</sup> Statistics</b>	<b>Level</b>	<b>Conc. (ng/mL)</b>	<b>Precision (% CV; RLU)</b>
			<b>Assay 1</b>   <b>Assay 2</b>
	PC 1	(b) (4)	
	PC 2	(b) (4)	
	PC 3	(b) (4)	
<b>Control Inter-Assay Statistics</b>	<b>Level</b>	<b>Conc. (ng/mL)</b>	<b>Precision (%CV; S/N)</b>
			(b) (4)
	PC 1	(b) (4)	
	PC 2	(b) (4)	
	PC 3	(b) (4)	
<b>Thawed Matrix Stability</b>	(b) (4)		
<b>Freeze-Thaw Stability</b>	(b) (4)		
<b>Drug Interference</b>	(b) (4)		
<b>Recovery</b>	Acceptable with ten out of ten sample lots meeting the acceptance criteria at (b) (4)		

<sup>a</sup> Inter-assay precision was assessed in two occasions (assays), the data from both assays are presented

2.9.7.2 What is NAb assay and its performance?

Patient samples confirmed positive in the confirmatory ADA assay were subjected to the NAb assay validated at (b) (4) in August 2013 under (b) (4) study MYY2. (b) (4)

(b) (4)

The NAb assay for detecting the presence of NAb to asfotase alfa was based (b) (4)

(b) (4)

Table 41 summarizes the validation results for the ADA assay. According to the CMC reviewer, the performance of the NAb is acceptable.

**Table 41. Summary of validation results for the NAb assay**  
(Source: Table 32 of Module 2.7.1 Summary of Biopharmaceutical Studies)

Parameter	Results Summary		
Method	(b) (4) (ICDIM 130 Version 1.00)		
Analyte	(b) (4)		
Matrix	Serum		
Sensitivity	(b) (4)		
PC anti-TNSALP (EP22 lot AA20120514)	(b) (4)		
PC Concentration	(b) (4)		
Control Intra-Assay Statistics	Level		Conc. (µg/mL) (%CV)
	PC Low 1	PC 1	(b) (4)
	PC Low 2	PC 2	(b) (4)
	PC High	PC 3	(b) (4)
Control Inter-Assay Statistics	Level		Conc. (µg/mL) Precision (%CV)
	PC Low 1	PC 1	(b) (4)
	PC Low 2	PC 2	(b) (4)
	PC High	PC 3	(b) (4)
Thawed Matrix Stability	(b) (4)		
Freeze/Thaw Stability	(b) (4)		
Drug Tolerance	(b) (4)		
Recovery	Acceptable with ten out of ten sample lots meeting the acceptance criteria (b) (4)		

### 3. LABELING

The underlined text is the proposed addition, while ~~the strikethrough text~~ is the proposed deletion. The changes recommended below are for the team's consideration. Additional modifications may be incorporated by the team in the final label.

#### 2.1 Dosage Regimen

The recommended dosage regimen of STRENSIQ for the treatment of infantile- and juvenile-onset HPP is 2 mg/kg of body weight administered subcutaneously three times per week, or a dosage regimen of 1 mg/kg of body weight administered subcutaneously six times per week.

## 2.3 Preparation

Caution: (b) (4) do not use the 80 mg/0.8 mL vial of STRENSIQ in pediatric patients (b) (4) [see Clinical Pharmacology (12.3)].

## 6.2 Immunogenicity

As with all therapeutic proteins, there is potential for immunogenicity. During clinical trials, anti-drug antibodies have been detected in patients receiving treatment with STRENSIQ using an electrochemiluminescent (ECL) immunoassay. Antibody positive samples were tested to determine the presence of neutralizing antibodies based on in vitro inhibition of the catalytic activity of STRENSIQ. Among (b) (4) patients with hypophosphatasia (HPP) patients enrolled in the clinical trials and who hadve post-baseline antibody data, (b) (4) tested positive for anti-drug antibodies at some time point after receiving STRENSIQ treatment. Among those (b) (4) patients, (b) (4) also showed the presence of neutralizing antibodies. No correlation was observed between the anti-drug antibodies titer and neutralizing antibodies (% inhibition) values (b) (4)

(b) (4) Formation of anti-drug antibody resulted in a reduced systemic exposure of asfotase alfa. [see Clinical Pharmacology (12.3)].

## 12.1 Mechanism of Action

HPP is caused by a deficiency in TNSALP enzyme activity, which leads to elevations in several TNSALP substrates, including PPi. Elevated extracellular levels of PPi block hydroxyapatite crystal growth which inhibits bone mineralization and causes an accumulation of unmineralized bone matrix which manifests as rickets and bone deformation in infants and children and as osteomalacia (softening of bones) once growth plates close along with muscle weakness (b) (4)

## 12.2 Pharmacodynamics

(b) (4) (b) (4) Infantile- and juvenile-onset HPP patients treated with (b) (4)-STRENSIQ had (b) (4)

reductions in plasma TNSALP substrates, PPi and PLP. Reductions in PPi and PLP levels did not correlate with clinical outcomes. The bone biopsy data from juvenile-onset HPP patients treated with STRENSIQ demonstrated decreases in osteoid volume and thickness indicating improved bone mineralization. (b) (4)

### 12.3 Pharmacokinetics



Based on data in 38 HPP patients, asfotase alfa pharmacokinetics (PK) exhibits dose proportionality across the dose range of 0.3 mg/kg to 3 mg/kg and appears to be time-independent. Steady state exposure was achieved as early as three weeks after the administration of the first dose.

Table summarizes the PK parameters at after subcutaneous administration of STRENSIQ at 2 mg/kg three times per week in Studies 2 and 3.

Table. Summary of Pharmacokinetic Parameters Following Subcutaneous Administration of STRENSIQ 2 mg/kg Three Times per Week

	Study 2	Study 3
N	(b) (4)	6
Age (years)	(b) (4)	(b) (4)
Weight at Baseline (kg)	(b) (4)	(b) (4)
t <sub>last</sub> (h)	(b) (4)	(b) (4)
t <sub>max</sub> (h)	(b) (4)	(b) (4)
C <sub>max</sub> (U/L)	(b) (4)	(b) (4)
AUC <sub>t</sub> (h*U/L)	(b) (4)	(b) (4)
C <sub>max,nor</sub> (U/L)/(U/kg)	(b) (4)	(b) (4)
AUC <sub>t,nor</sub> (h*U/L)/(U/kg)	(b) (4)	(b) (4)
Accumulation Ratio <sup>a</sup>	(b) (4)	(b) (4)

<sup>a</sup>Ratio values reflect the fold increase of AUC<sub>t</sub> from Week 1 based on mean AUC<sub>t</sub> values.

Data are presented as mean ± standard deviation.

t<sub>last</sub>, time of last concentration; t<sub>max</sub>, time of maximal concentration; C<sub>max</sub>, maximal concentration; AUC<sub>t</sub>, area under the concentration-time curve over a dosing interval of 48 hours; C<sub>max,nor</sub>- (b) (4)

Formation of anti-drug antibody resulted in a reduced systemic exposure of asfotase alfa (b) (4)

End of Document

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# OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

## 1 SUMMARY OF FINDINGS

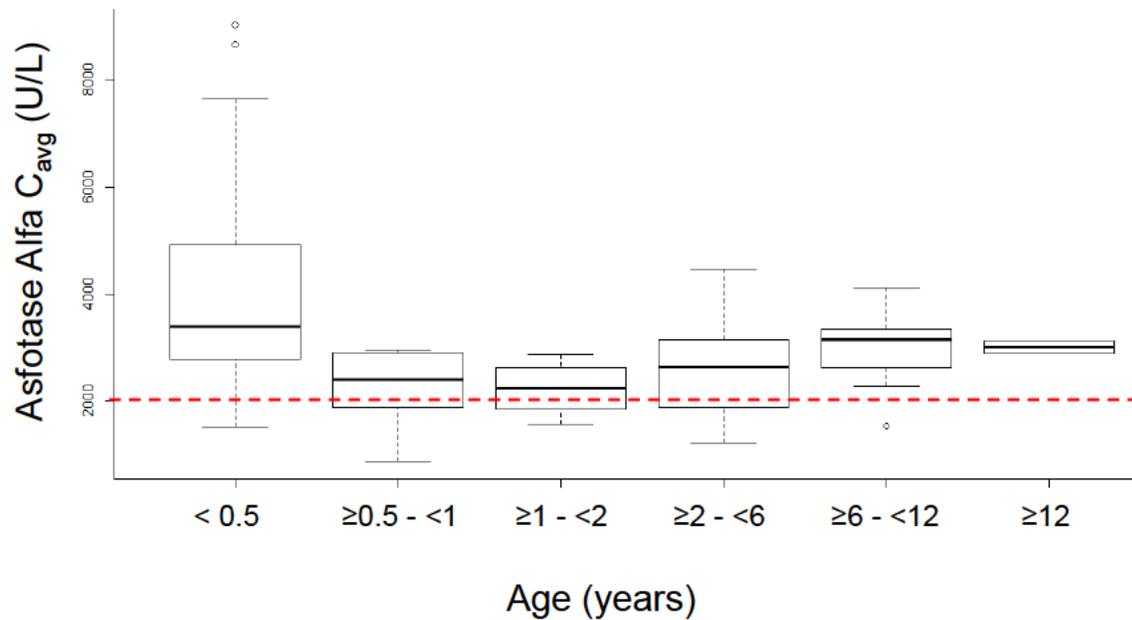
### 1.1 Key Review Questions

The purpose of this review is to address the following key questions.

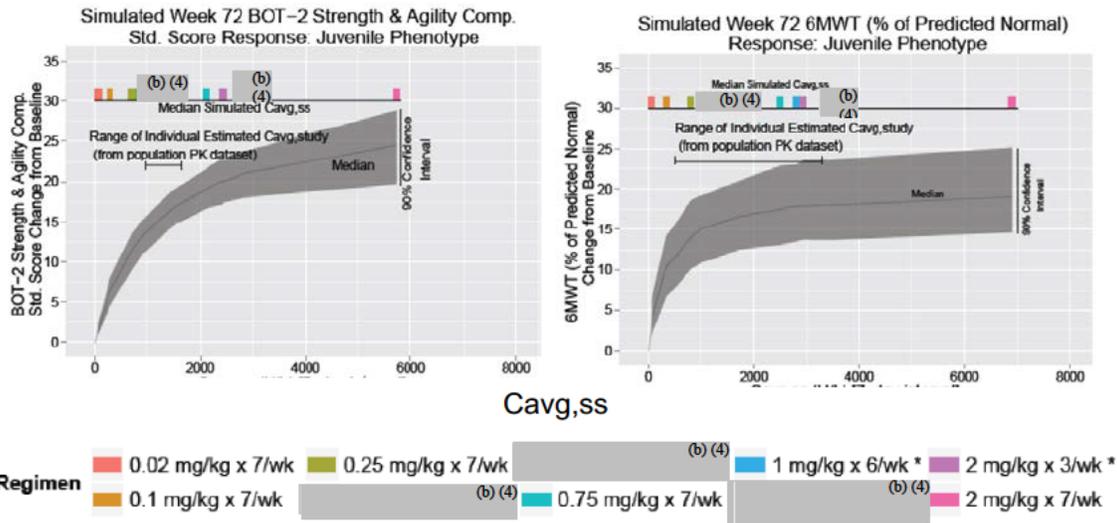
#### 1.1.1 Do the exposure-response relationships support the proposed 6 mg/kg weekly dose?

Yes, the dose of 6 mg/kg produces average concentrations (Figure 1) that appear to fall in the plateau of the exposure-response curves for MPOMA-G gait analysis, BOT-2 score (Figure 2), 6 minute-walk test (Figure 2), PPI concentrations (Figure 3), and PLP concentrations (Figure 3).

**Figure 1. Average Asfotase Alfa Concentration versus Age. Asfotase Alfa C<sub>avg</sub> was calculated from the individual post hoc Bayesian estimates of clearance for each individual, and the average dose received over the duration of treatment.**

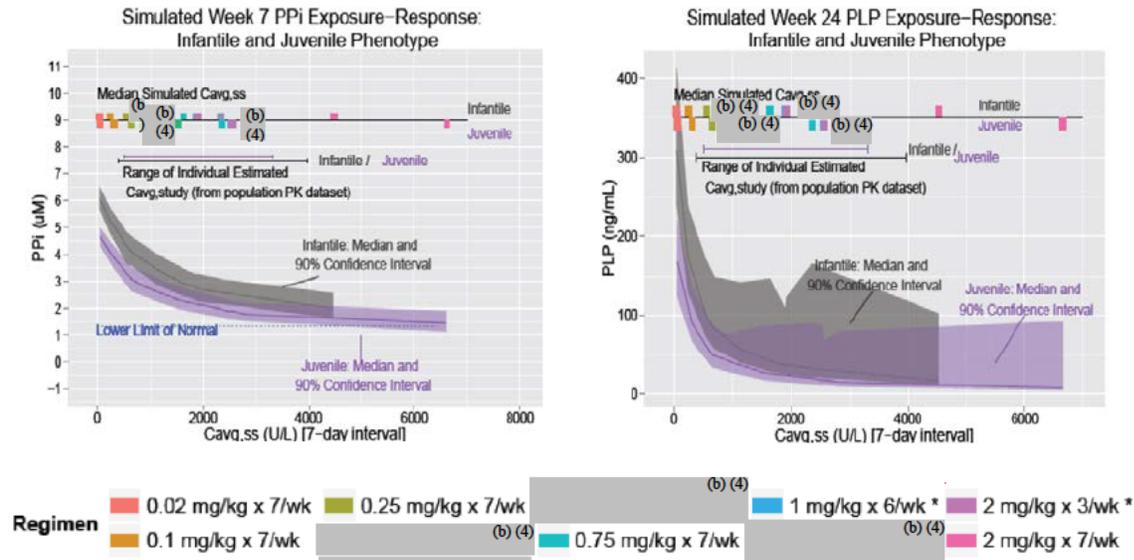


**Figure 2. Exposure Response Relationships for BOT-2 Score and 6-Minute Walk Distance at Week 72.**



(Source: Applicant’s Population PK Report, Figure 4)

**Figure 3. Exposure Response for PPI (Week 7) and PLP (Week 24) Concentrations.**

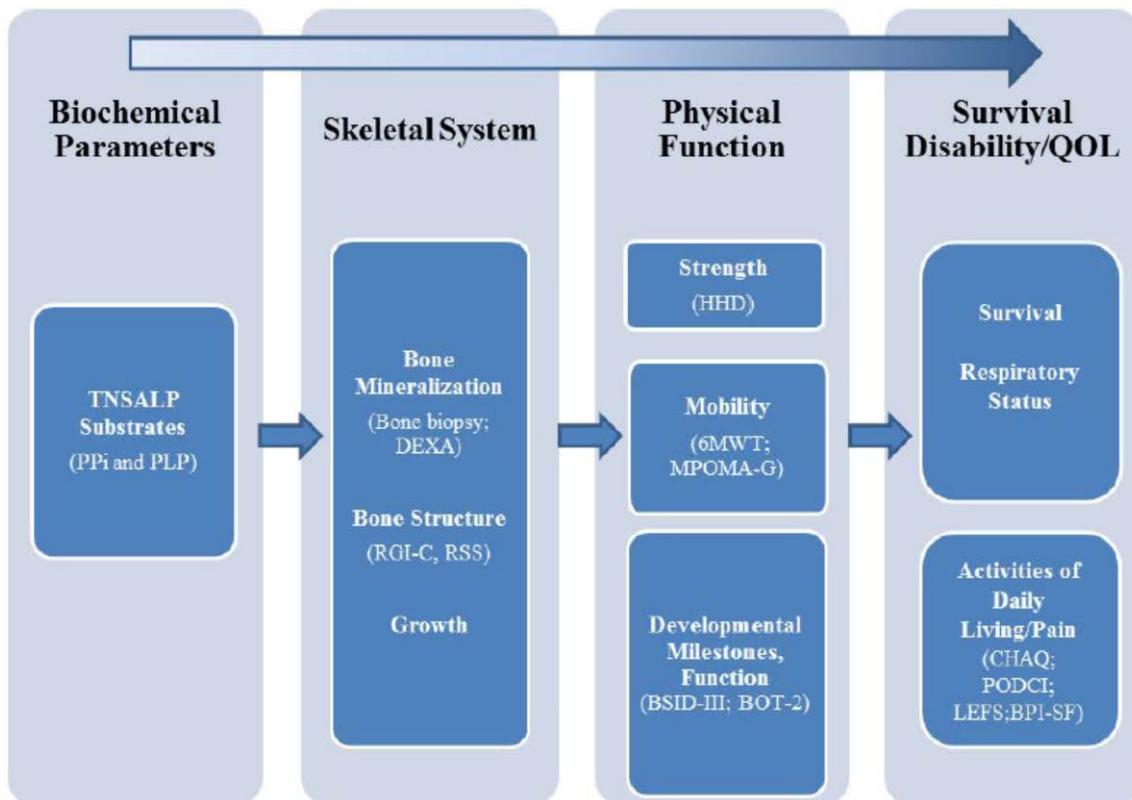


(Source: Applicant’s Population PK Report, Figure 4)

The applicant’s approach to assessing efficacy in this rare disease has been multi-faceted in terms of efficacy measures. Their original pre-specified primary endpoint was the radiographic assessment of bone structure. However, the agency agreed endpoints were overall survival as compared to historical controls for infantile onset patients and a measure of mobility by gait analysis (MPOMA-G Score) was as compared to that for historical controls for the juvenile onset population. The spectrum of measurements made by the applicant is shown in Figure 4. Additionally, there has been focus by the review team on bone-biopsy and growth as well. The established exposure-response relationships described above and below collectively are important evidence of effectiveness across the continuum of measures between pharmacological action of the drug and improvement on the quality of life and patient survival. Given the

limitations of drug development in rare diseases, that there are relatively few patients in a few studies that evaluated different endpoints, it is crucial to consider the totality of these relationships together.

**Figure 4. Endpoints evaluated in Clinical Studies of Patients with Infantile- or Juvenile-onset Hypophosphatasia**

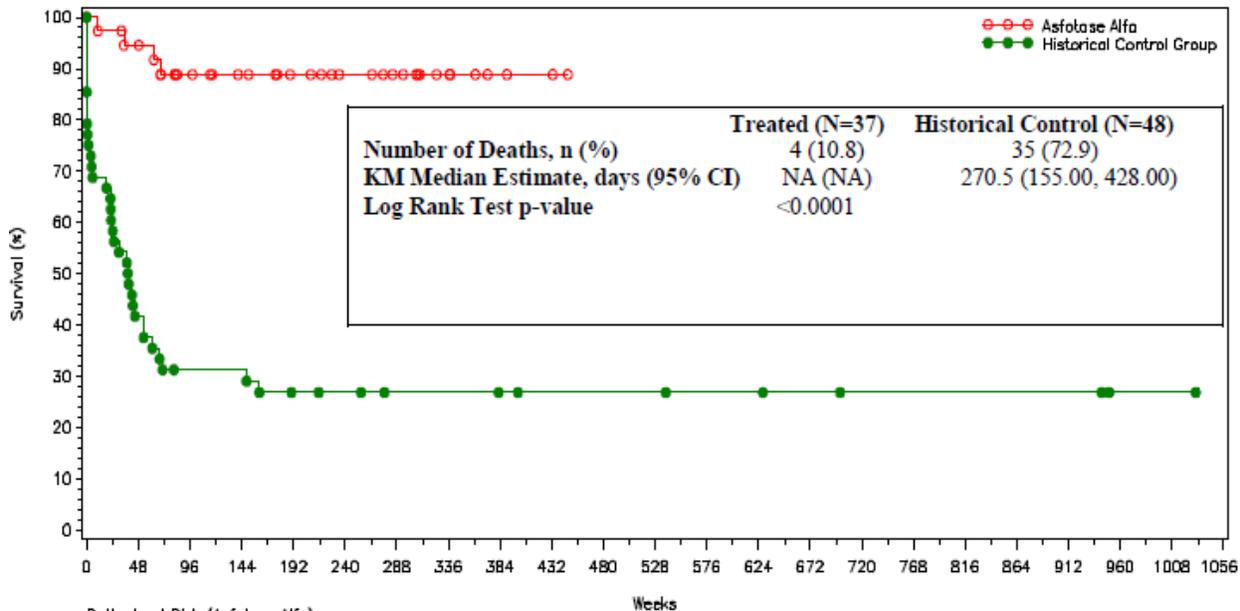


Abbreviations: 6MWT = 6-Minute Walk Test; BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; BPI-SF = Brief Pain Inventory-Short Form; BSID-III = Bayley Scales of Infant and Toddler Development, Third Edition; CHAQ = Child Health Assessment Questionnaire; DEXA = dual energy x-ray absorptiometry; HHD = hand-held dynamometry; HPP = hypophosphatasia; LEFS = Lower Extremity Functional Scale; PODCI = Pediatric Outcomes Data Collection Instrument; PLP = pyridoxal-5' -phosphate; POMA-G = Performance-Oriented Mobility Assessment, Gait subtest; PPi = inorganic pyrophosphate; QOL = quality of life; RGI-C = Radiographic Global Impression of Change; RSS = Rickets Severity Scale.  
 Note: Bone biopsy, DEXA and assessments of disability and quality of life were performed in Studies ENB-006-09/ENB-008-10 and ENB-009-10 (ie, in patients  $\geq 5$  years of age); video recordings of the 6MWT obtained as part of Studies ENB-006-09/ENB-008-10 were used as the basis for evaluating gait in treated patients, but the scoring itself was only performed for the summary of clinical efficacy presented in this document.

(Source: Applicant's Summary of Clinical Efficacy, Figure 2)

Exposure response analysis for overall survival in the infantile-onset population indicated there was a significant relationship ( $p < 0.0041$  with a hazard ratio of 0.9999 per U/L change in exposure). However these survival data were sufficiently limited to suggest a minimum/target exposure of asfotase alfa. There were 4 deaths on treatment and 35 in the historical control population. Kaplan-Meier curves for this comparison are shown in Figure 5.

**Figure 5. Overall Survival in Asfotase Alfa-Treated versus Historical Control Patients with Infantile-Onset HPP.**



(Source: Applicant’s Summary of Clinical Efficacy, Figure 13)

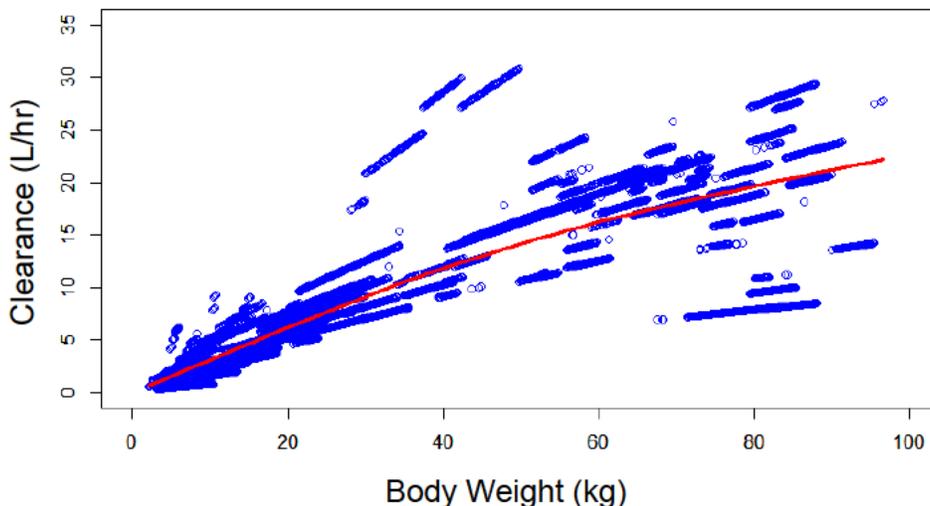
Exposure-response analysis was performed for MPOMA-G score in eight patients with juvenile-onset HPP that received asfotase alfa and in an additional six historical controls. A linear mixed effects analysis was performed on the rate of change in MPOMA-G score as a function of asfotase alfa  $C_{average}$ . Results indicated a small increase in the MPOMA-G score per day = 0.00076 or 0.28 per year. Given this slight change, limited numbers, and variable assessment of the score from 6MWT videos, this endpoint was given limited value by the review team in assessing the efficacy of asfotase alfa. That being said the model did establish an EC50 value of 1620 U/L which is consistent with the other established exposure response relationships.

The reviewer’s exposure-response analysis for growth indicated no clear relationship between Z-score for height and asfotase alfa  $C_{average}$  based on the average dose and average clearance of the individual over the duration of their treatment (See Section 4.4.3 for further details). Thus growth as indicated by Z-score for height may not be an endpoint that is considered evidence of effectiveness.

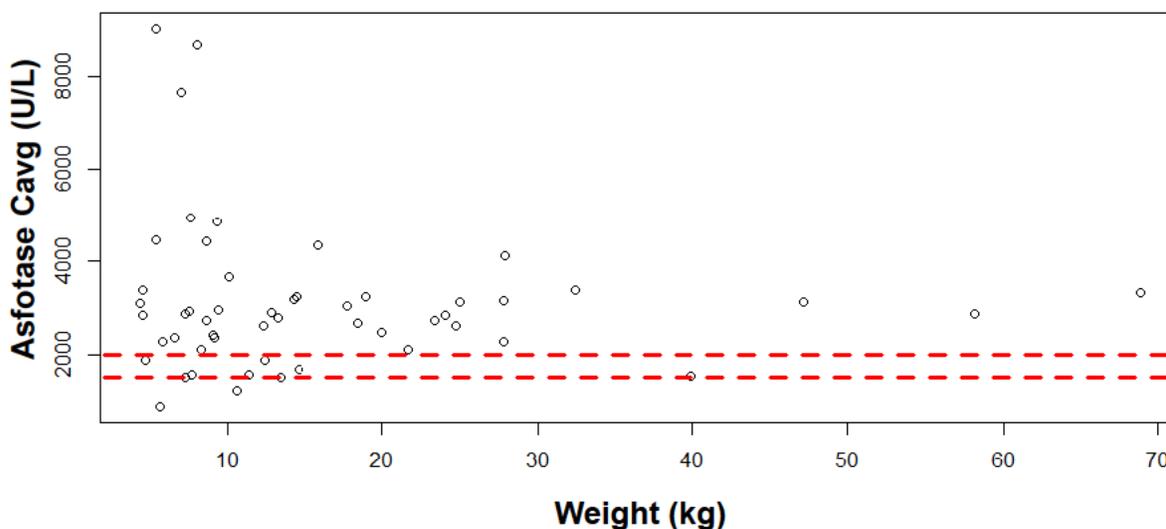
### 1.1.2 Is weight-based dosing (6 mg/kg/week) appropriate?

Yes, dosing by body weight produces similar exposures across the range of age (Figure 1) and thus weight (weight and age are correlated in the HPP population). The underlying relationship between clearance and body-weight is shown in Figure 6 and supports weight based dosing. Figure 7 depicts the projected exposures at the proposed dose based on the patient’s Bayesian post hoc population PK estimate for clearance.

**Figure 6. Clearance is dependent on body weight. Individual Bayesian post hoc estimates of clearance from the final population PK model are plotted against the patient’s body weight (blue circles). The modeled relationship between clearance and body weight is depicted by the red line.**



**Figure 7. Body weight based dosing would produce similar exposures across the range of patient weights. Asfotase Cavg was calculated using each patient’s Bayesian post hoc estimate of clearance and the proposed dose of 6 mg/kg/week and are shown by the open circles. The red dashed lines indicate the minimum therapeutic target based on the established exposure response relationships.**



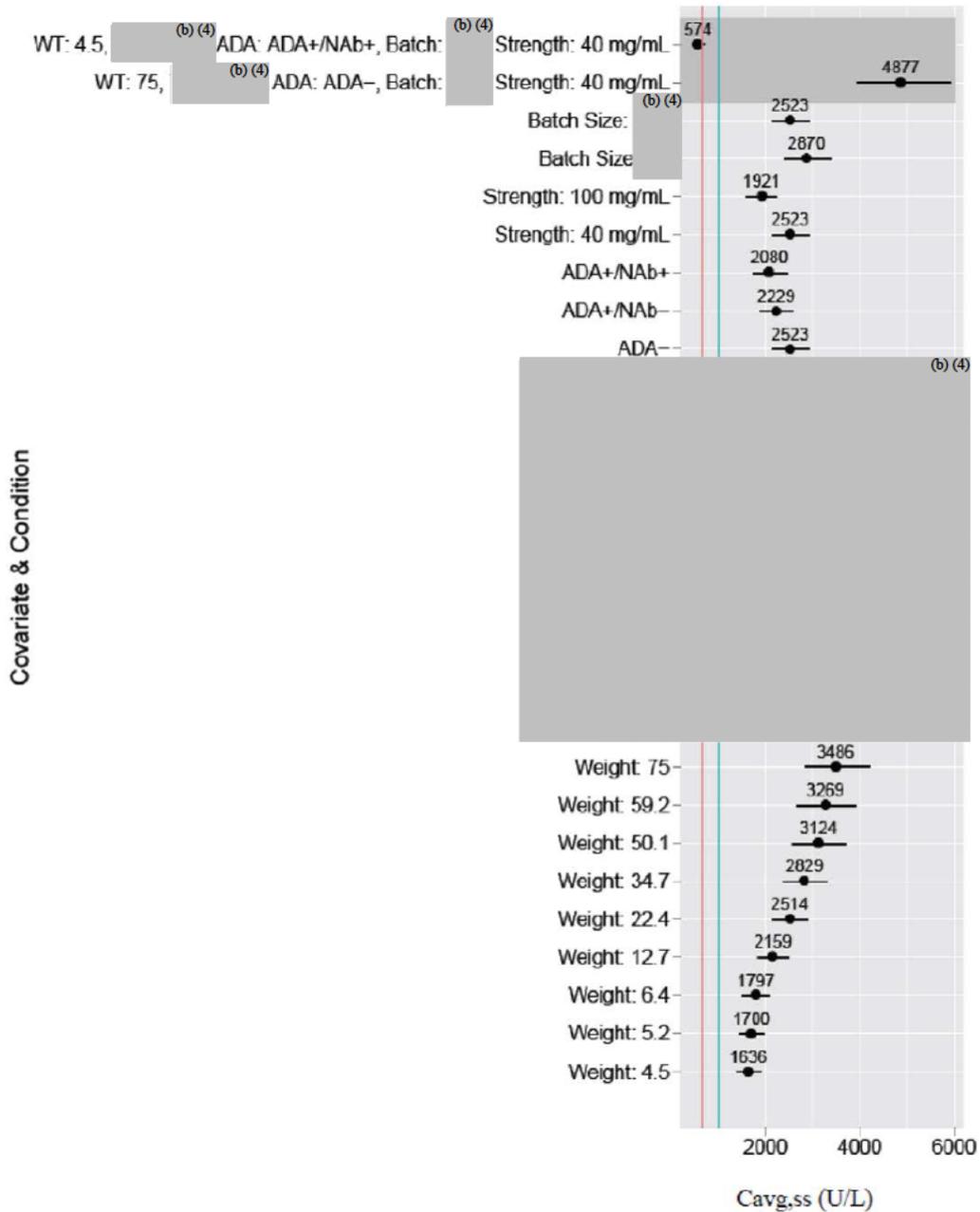
**1.1.3 Should the batches of asfotase alfa be restricted to reduce the range (b) (4) that patients will receive?**

Yes, between the extremes (b) (4) studied there was approximately a three-fold change in the clearance (Figure 22) and  $C_{average}$  (Figure 8) of Asfotase Alfa. (b) (4)

(b) (4)



**Figure 8. Impact of Different Covariates and their Combinations on Simulated Cavg,ss**



(Source: Applicants Response to FDA Information Request, 03 Feb 2015, Figure 4)

Discussions with the Office of Biotechnology Products are ongoing to establish a path forward with the applicant regarding the control of (b) (4) in the product and implications for post marketing commitments.

**1.1.4 Should infantile-onset patients have the option to increase their dose based on lack of response to treatment at the 6 mg/kg/week dose?**

Yes, the option to increase the dose in patients who are not responding to the 6 mg/kg dose is acceptable from an exposure-response perspective.

The medical review by Dr. Carla Epps has suggested that infants that increased their dose from 6 mg/kg/week to  $\geq 9$  mg/kg/week appeared to gain additional treatment benefit.

Exposure response relationships for overall survival, MPOMA-G, TNSALP substrates PPI and PLP, BOT-2 score, and 6-MWT suggest that increasing exposure of asfotase alfa may offer additional patient benefit. However, it does appear that of the few infants that increased their dose and exhibited improvement that their mean exposures were in the upper 75<sup>th</sup> percentile. Their higher exposures may be in part due to underdeveloped clearance of asfotase alfa in patients that are less than 6 months of age as the majority of these patients were less than 6 months old.

Despite these higher exposures in the infants that showed benefit, inherent variability in disease progression and response to treatment may cause the ideal exposure for each patient to vary. Thus, for a patient population with a high unmet medical need, low risk of adverse events with asfotase alfa, (b) (4) it appears reasonable to increase the dose 50% in infants who are not responding to asfotase alfa at the 6 mg/kg/week dose.

## **2 PERTINENT REGULATORY BACKGROUND**

Asfotase Alfa is a new molecular entity being developed for the treatment of infantile-onset and juvenile-onset hypophosphatasia (HPP). HPP is a genetic deficiency of tissue non-specific alkaline phosphatase (TNSALP) which is responsible for bone mineralization and the clearance of substrates inorganic pyrophosphate (PPI), Pyridoxal-5'-Phosphate (PLP), and phosphoethanolamine (PEA). Asfotase Alfa is a fusion protein of recombinant human TNSALP, human IgG1 Fc, and a polyaspartate region. The agency agreed primary endpoints were overall survival for the infantile-onset population compared to historical controls and the MPOMA-G assessment of physical motor function compared to historical controls for the juvenile-onset population. While statistically significant benefit was observed for asfotase alfa treated patients compared to historical controls for these endpoints, the numbers of patients are few and exposure-response relationships for other endpoints are presented as additional supportive evidence of effectiveness.

## **3 RESULTS OF SPONSOR'S ANALYSIS**

### **3.1 Clinical Trials:**

There were a total of seven different clinical trials with asfotase alfa. However, two of those trials were extensions of prior studies. Of these the largest trial was ENB-010-10 which enrolled 28 patients. In total 77 patients enrolled in the asfotase alfa studies and 69 completed the trials. PK data were available from 73 of these subjects. Thus, the population PK and PK/PD models included data from across these studies.

**Table 1. Clinical Trials with Asfotase Alfa.**

Descriptor	Study Number				
	ENB-001-08 <sup>a</sup>	ENB-002-08/ ENB-003-08 <sup>b</sup>	ENB-010-10	ENB-006-09/ ENB-008-10 <sup>c</sup>	ENB-009-10
Study Design	Multicenter, multinational, open-label, dose-escalation, safety & efficacy, PK, PD	Multicenter, multinational, open-label, single-group assignment, safety & efficacy, PK, with extension	Multicenter, multinational, open-label, safety & efficacy, PK	Multicenter, multinational, open-label, dose-comparison, parallel-assignment, historical control, safety & efficacy, PK, PD, with extension	Randomized, multicenter, multinational, open-label, dose-ranging, concurrent control, safety & efficacy, PK
Countries	Canada, USA	Canada, UAE, UK, USA	Canada, Germany, Japan, Taiwan, Turkey, USA	Canada, USA	Canada, USA
Study Status	Completed	Completed/ Ongoing	Currently enrolling and ongoing	Completed/ Ongoing	Ongoing
Number of Patients Enrolled	6	11/10	28 <sup>d</sup>	13/12	19
Number of Patients Completed <sup>a</sup>	6	10/9 <sup>a</sup>	24 <sup>a,f</sup>	12/12 <sup>a</sup>	18 <sup>a</sup>
Age at Inclusion	24 to 58 years	0.5 to 35 months	0 to 72 months <sup>e</sup>	5 to 12 years	13 to 66 years
Number of Patients by Age at Disease Onset (age at onset of first signs and/or symptoms of HPP) <sup>d</sup>					
Pediatric Onset	4 <sup>a</sup>	11	28	13	16 <sup>a</sup>
Infantile (<6 months)	1	11	28	5	4
Juvenile (≥6 months to <18 years)	3	0	0	8	12
Adult Onset (≥18 Years)	0	0	0	0	2
Unknown Onset	2	0	0	0	1
Included in Integrated Efficacy Analysis	No	Yes	Yes	Yes	Yes
Descriptor	Study Number				
	ENB-001-08 <sup>a</sup>	ENB-002-08/ ENB-003-08 <sup>b</sup>	ENB-010-10	ENB-006-09/ ENB-008-10 <sup>c</sup>	ENB-009-10
Dosing Regimen	<b>Cohort 1:</b> Single 3 mg/kg IV dose followed by 1 mg/kg SC dose 1×/wk for 3 wks <b>Cohort 2:</b> Single 3 mg/kg IV dose followed by 2 mg/kg SC dose 1×/wk for 3 wks	Single 2 mg/kg IV dose followed by 1 mg/kg SC 3×/wk, with dose adjustments per protocol	2 mg/kg SC 3×/wk or 1 mg/kg SC 6×/wk, with dose adjustments per protocol	2 mg/kg SC 3×/wk or 3 mg/kg SC 3×/wk, with dose adjustments per protocol/ 1 mg/kg SC 3×/wk later changed to 2 mg/kg SC 3×/wk or 1 mg/kg SC 6×/wk, with dose adjustments per protocol	No treatment (control) or 0.3 mg/kg SC QD or 0.5 mg/kg SC QD <b>Extension 1:</b> 0.5 mg/kg SC QD starting at Week 24 <b>Extension 2:</b> 1.0 mg/kg SC 6×/wk
Analysis cutoff date for CSR	NA	16 NOV 2012	22 NOV 2013	22 JAN 2013	29 JAN 2013
Analysis cutoff date for Integrated Analyses	NA	22 NOV 2013	22 NOV 2013	05 NOV 2013	30 OCT 2013

Abbreviations: CSR = clinical study report; HPP = hypophosphatasia; IV = intravenous; NA = not applicable; PD = pharmacodynamic; PK = pharmacokinetic; QD = once daily; SC = subcutaneous; UAE = United Arab Emirates; UK = United Kingdom; USA = United States of America; wk(s) = week(s).

<sup>a</sup> Study ENB-001-08 is not included in the pooled efficacy and safety analyses; results from this study are discussed separately. Four of the 6 patients who enrolled in ENB-001-08 also enrolled in ENB-009-10 and are only counted once.

<sup>b</sup> Study ENB-003-08 is the extension study for Study ENB-002-08. Patients who rolled over from the parent study to the extension study were only counted once.

<sup>c</sup> Study ENB-008-10 is the extension study for Study ENB-006-09. Patients who rolled over from the parent study to the extension study were only counted once.

<sup>d</sup> Study ENB-010-10 is continuing to enroll patients.

<sup>e</sup> For ongoing studies, the number of completed patients is reflective of the number of patients that were continuing asfotase alfa treatment as of the analysis cutoff date for the integrated analyses.

<sup>f</sup> As of the data cutoff date for the integrated analyses, 3 treatment-emergent deaths were reported in ENB-010-10; one additional death pre-treatment was reported. Refer to Section 2.7.4.7.1 for late-breaking deaths reported in ENB-010-10 (cutoff date 21APR 2014).

<sup>g</sup> Patient 010-01-07 was 1 day old at first dose of asfotase alfa.

(Source: Applicant’s Summary of Clinical Efficacy, Table 2)

### 3.2 Population PK Analysis as of December 2014 (Original BLA Submission):

Data representing the complete clinical course for all individuals, with respect to the repeated measures PK and pharmacodynamic (PD) endpoints, were assembled for analyses. In addition to PK and PD endpoints, the entire dosing history (amount and frequency), including lot (b) (4) (b) (4) and lot potency was represented, along with covariate factors such as age, weight, clinical laboratory values, neutralizing antibody status, and disease phenotype.

Population PK and pharmacokinetic-pharmacodynamic (PK-PD) analyses for repeated-measures endpoints were conducted via nonlinear mixed effects modeling. Population PK data were

described with a linear two-compartment model, and population PD data (for all efficacy endpoints) were described using indirect PD response models using time-continuous patient-specific predicted serum asfotase alfa activity. A full covariate model was constructed given pre-specified covariates and some exploratory variables with care to avoid correlation or collinearity in predictors. The resulting models were evaluated for goodness of fit and qualified. Model-based simulations were conducted to explore the dose-exposure-response relationships for all endpoints.

(Source: Applicant's Population PK Report, Methods Section)

**The applicant's final population PK model parameters are shown in Table 2.**

**Table 2. Asfotase-Alfa Final Population PK Model Parameter Estimates**

PK Parameter (Unit)*	NONMEM Parameter*	Estimate	95%CI**
$V_{2,70\text{kg}}$ (L)	$\exp(\theta_2)$	3.93	(b) (4)
$V_{3,70\text{kg}}$ (L)	$\exp(\theta_3)$	46.1	(b) (4)
$Q_{70\text{kg}}$ (L/day)	$\exp(\theta_4)$	51.9	(b) (4)
$k_a$ (day <sup>-1</sup> )	$\exp(\theta_5)$	0.625	(b) (4)
$F$	$\exp(\theta_6)$	0.571	(b) (4)
ALAG (day)	$\exp(\theta_7)$	0.144	(b) (4)
CL~ADA+/NAb-	$\exp(\theta_8)$	1.11	(b) (4)
CL~ADA+/NAb+	$\exp(\theta_9)$	1.21	(b) (4)
$k_a \sim \text{Batchsize}$	$\exp(\theta_{11})$	0.930	(b) (4)
$F \sim \text{Batchsize}$	$\exp(\theta_{12})$	0.910	(b) (4)
CL~AllometricExponent	$\theta_{13}$	0.776	(b) (4)
IVvar CL ( $\omega_{CL}^2$ )	$\Omega_{1,1}(\eta_1)$	0.194 (%CV=46.3)	(b) (4)
IVcov CL, $V_2$ ( $\omega_{CL}\omega_{V_2}$ )	$\Omega_{2,1}$	-0.0180	(b) (4)
IVvar $V_2$ ( $\omega_{V_2}^2$ )	$\Omega_{2,2}(\eta_2)$	0.550 (%CV=85.7)	(b) (4)
IVcov CL, $V_3$ ( $\omega_{CL}\omega_{V_3}$ )	$\Omega_{3,1}$	0.0235	(b) (4)
IVcov $V_2, V_3$ ( $\omega_{V_2}\omega_{V_3}$ )	$\Omega_{3,2}$	0.299	(b) (4)
IVvar $V_3$ ( $\omega_{V_3}^2$ )	$\Omega_{3,3}(\eta_3)$	0.171 (%CV=43.2)	(b) (4)
IVcov CL, $k_a$ ( $\omega_{CL}\omega_{k_a}$ )	$\Omega_{4,1}$	-0.132	(b) (4)
IVcov $V_2, k_a$ ( $\omega_{V_2}\omega_{k_a}$ )	$\Omega_{4,2}$	-0.0154	(b) (4)
IVcov $V_3, k_a$ ( $\omega_{V_3}\omega_{k_a}$ )	$\Omega_{4,3}$	-0.0773	(b) (4)
IVvar $k_a$ ( $\omega_{k_a}^2$ )	$\Omega_{4,4}(\eta_4)$	0.768 (%CV=108)	(b) (4)
Res <sub>additive</sub> ( $\sigma_{\text{additive}}^2$ )	$\Sigma_{1,1,\text{additive}}(\epsilon_1)$	0.139 (SD=0.4)	(b) (4)

(Source: Applicant's Population PK Report, August 2014, Table 30)

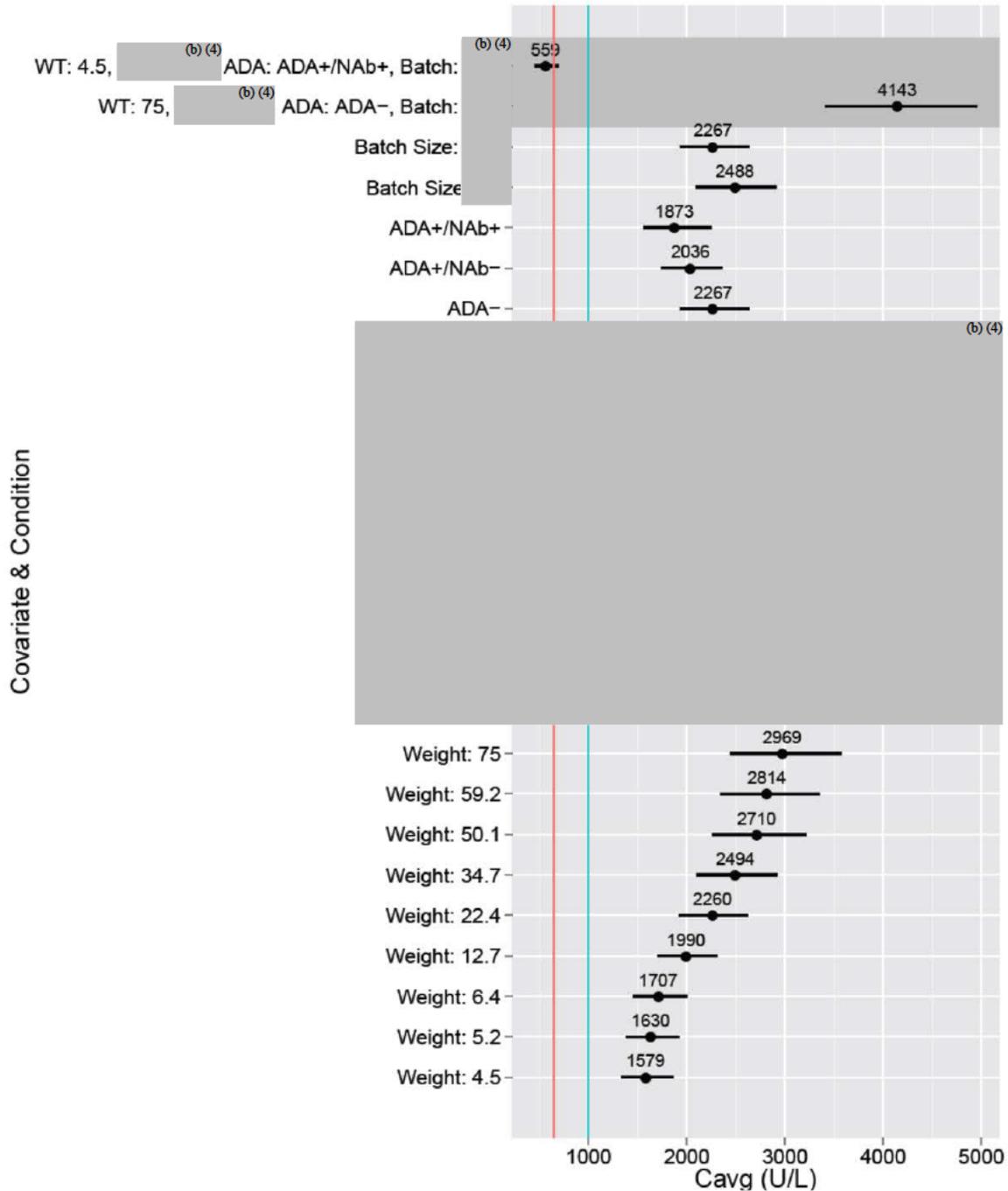
The model covariate structure is denoted by the following equations:

(b) (4)

(b) (4)

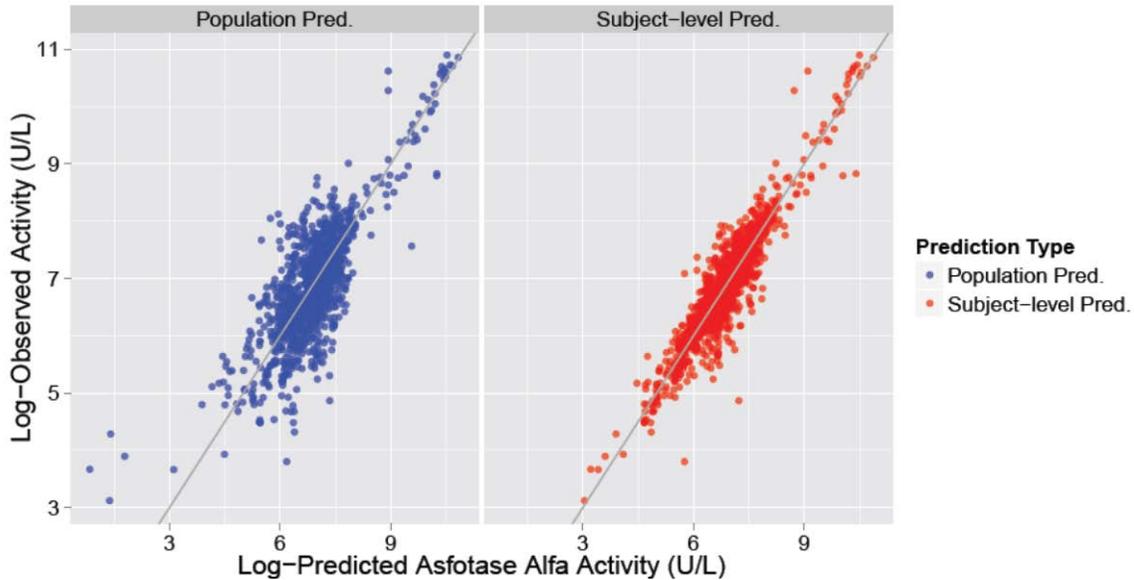
The range of effect of each covariate in the final population PK model is shown in Figure 8 by simulated asfotase alfa Cavg values.

**Figure 9. Effect of Population PK Covariate on Asfotase Alfa  $C_{avg}$ . Simulated  $C_{avg}$  values are based on a regimen of 2 mg/kg given three times per week. Dose activity is assumed to be 990 U/mg. Covariates were fixed at the following values, except when the subject of perturbation: <sup>(b) (4)</sup> weight, 22.7 kg; anti-drug and neutralizing antibodies, negative; and batch size, <sup>(b) (4)</sup>. The blue and red lines depict the applicant's targeted exposure based on non-clinical efficacy studies (see the Reviewer's Comments).**



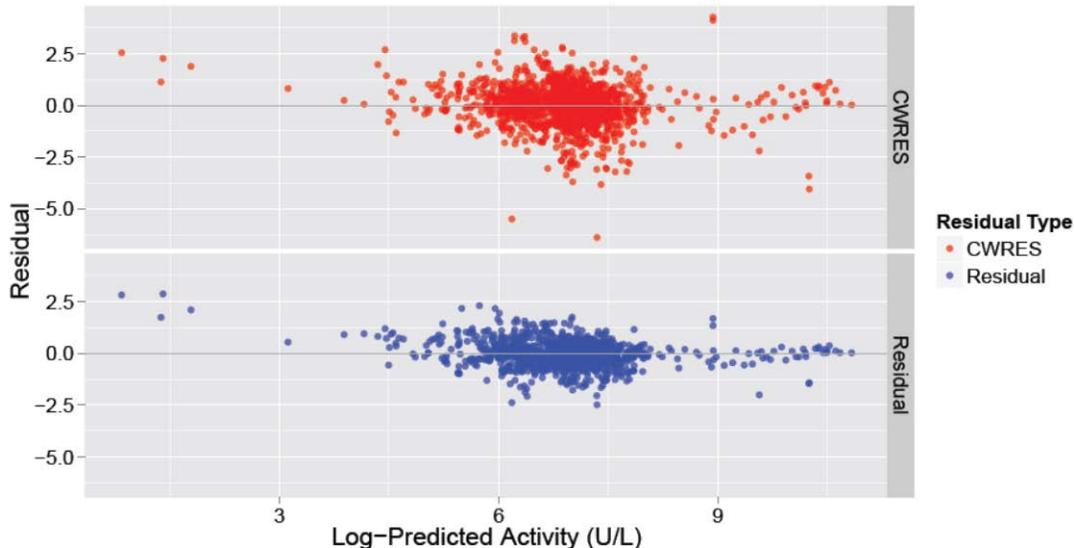
Population PK model diagnostic plots are shown in Figure 9 through Figure 12.

**Figure 10. PK: Log-Observed vs. log-population and log-individual predicted for final population PK model. Log-Observed asfotase alfa activity (U/L) data are plotted vs. log-population (blue) and individual (red) predictions for the final population PK model. Values are indicated by circles. The line of identity (solid grey) is included as reference.**



(Source: Applicant's Population PK Report, August 2014, Figure 20)

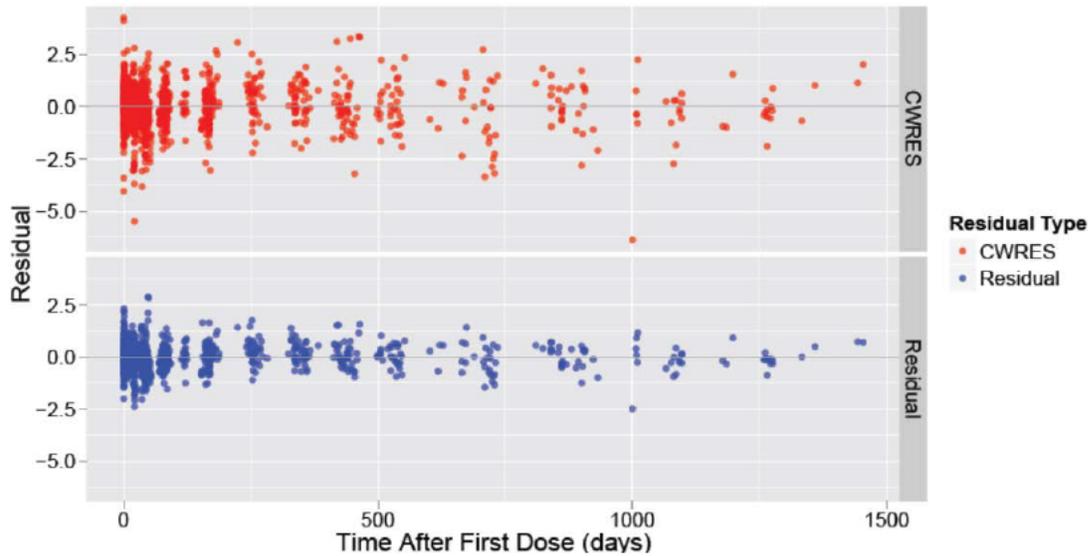
**Figure 11. PK: Residuals vs. log-population predicted asfotase alfa activity for final population PK Model. Residuals (blue) and conditional weighted residuals (red) are plotted vs. log-population predicted asfotase alfa activity (U/L) for the final population PK model. Values are indicated by circles. The solid grey line is a reference line for the value of zero.**



(Source: Applicant's Population PK Report, August 2014, Figure 23)

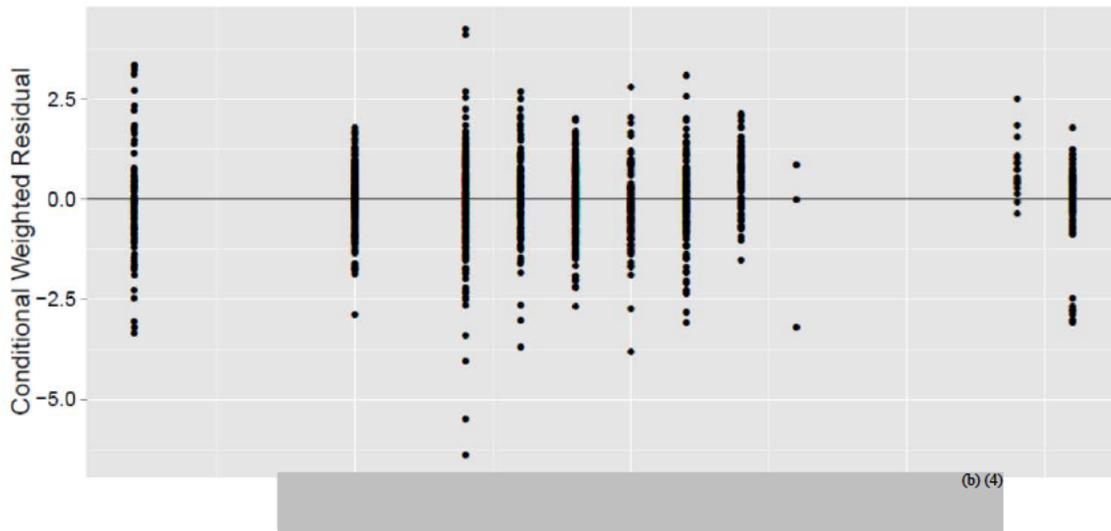
**Figure 12. PK: Residuals vs. time after first dose for final population PK model. Residuals (blue) and conditional weighted residuals (red) are plotted vs. log-population predicted**

asfotase alfa activity (U/L) for the final population PK model. Values are indicated by circles. The solid grey line is a reference line for the value of zero.



(Source: Applicant's Population PK Report, August 2014, Figure 24)

**Figure 13. Conditional weighted residuals (CWRES) versus (b) (4) from the final population PK model. CWRES are plotted versus (b) (4). Values are indicated by closed circles. A solid line CWRES reference line is included at zero.**



(Source: Applicant's Population PK Report, August 2014, Figure 30)

**Reviewer's Comments:**

The sponsor's population PK model appears reasonable to indicate that both body weight and (b) (4) play an important role in asfotase alfa exposure. However, the model was unable to suggest that the youngest infants actually exhibit higher exposures compared to patients > 6 months of age for the same weight based dosing regimen (See the reviewer's analysis for further details). The effect of (b) (4) on clearance was independently confirmed by the reviewer.

Regarding the applicant's target exposures in Figure 8, the reviewer does not agree that these are the target exposure for clinical efficacy. Both the left and right panels of Figure 2 and Figure 3 based on clinical data suggest exposures of 2000 U/L would place the exposure in the plateau of maximal response.

**3.3 Population PK Analysis as of February 2015 (Response to FDA Information Request):**

In January, 2015 the agency requested the applicant: **“perform population pharmacokinetic (PK) analysis with formulation strength (i.e., 40 mg/mL and 100 mg/mL) as a covariate to assess the impact of formulation strength on asfotase alfa PK and pharmacodynamics (PD). Submit the revised population PK and PK-PD datasets, and the results from the updated population PK and PK-PD analyses.”**

The applicant subsequently updated their population PK model with formulation strength included as a covariate on both Ka and bioavailability.

The resulting Pop-PK model parameter estimates and 95% confidence intervals (CI) are presented in Table 3. The estimate (90% CI) for the relative ratio of 100 mg/mL to 40 mg/mL formulation strength for absorption rate constant was (b) (4) and for relative bioavailability was (b) (4). These indicate that the effect of formulation strength on absorption rate constant was not differentiable from the null value (eg, ratio of 1). The effect of formulation strength on relative bioavailability was different from the null value, with a point estimate indicating an approximate (b) (4) decrease in bioavailability for the 100 mg/L formulation strength. Both effects were estimated with moderate precision. Compared to the Pop-PK model without formulation strength as covariate, the model with formulation strength as covariate had a lower objective function by 23.893 units. The estimates of all other parameters were similar to the model without the formulation strength as covariate, as reported in the submission (Table 2).

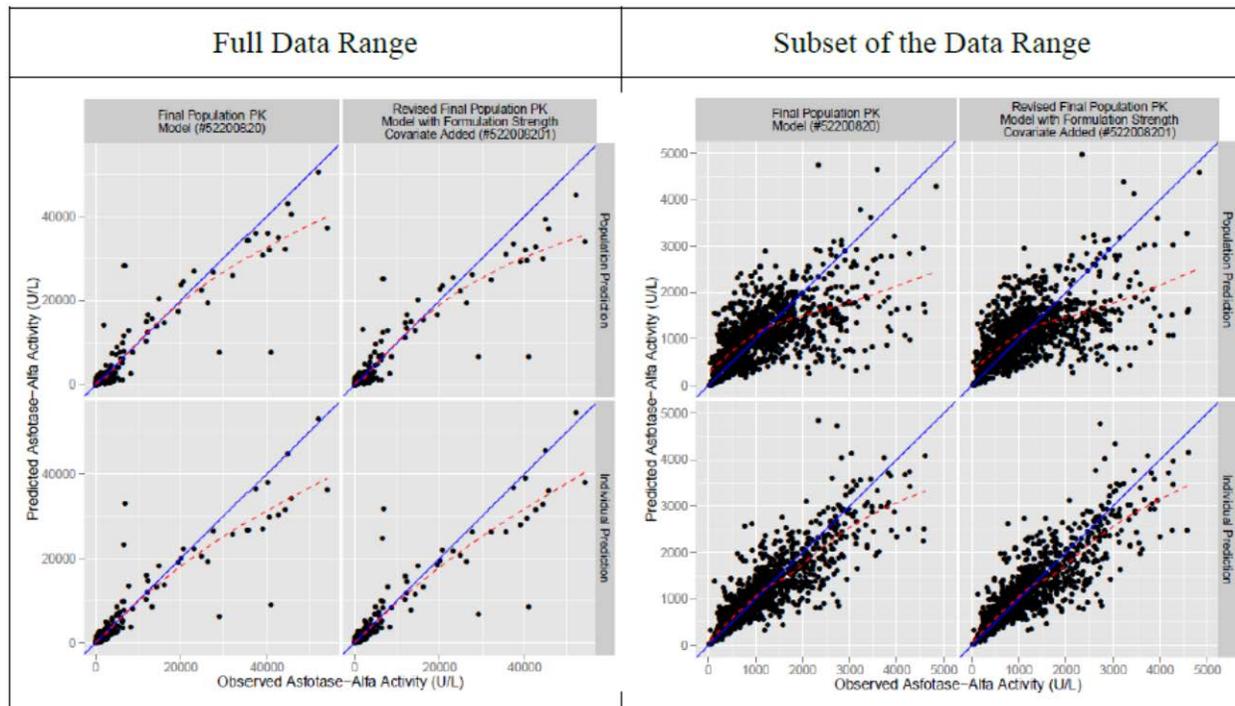
(Source: Applicants Response to FDA Information Request, 03 Feb 2015, page 4)

**Table 3. Asfotase Alfa Population PK Model Parameter Estimates from the Revised Model that included Formulation Strength as a Covariate on Ka and F.**

PK Parameter (Unit)*	NONMEM Parameter*	Estimate	95%CI**
$V_{2,70\text{kg}}$ (L)	$\exp(\theta_2)$	4.55	(b) (4)
$V_{3,70\text{kg}}$ (L)	$\exp(\theta_3)$	44.6	(b) (4)
$Q_{70\text{kg}}$ (L/day)	$\exp(\theta_4)$	52.9	(b) (4)
$k_a$ (b) (4) 40mg/mL (day <sup>-1</sup> )	$\exp(\theta_5)$	0.662	(b) (4)
$F$ (b) (4) 40mg/mL	$\exp(\theta_6)$	0.620	(b) (4)
ALAG (day)	$\exp(\theta_7)$	0.140	(b) (4)
CL ~ ADA + /NAb-	$\exp(\theta_8)$	1.13	(b) (4)
CL ~ ADA + /NAb+	$\exp(\theta_9)$	1.22	(b) (4)
$k_a$ (b) (4) ~ Batchsize (b) (4)	$\exp(\theta_{11})$	0.881	(b) (4)
$F$ (b) (4) ~ Batchsize (b) (4)	$\exp(\theta_{12})$	0.880	(b) (4)
CL ~ AllometricExponent	$\theta_{13}$	0.733	(b) (4)
$k_a$ , 40mg/mL ~ FormulationStrength <sub>100mg/mL</sub>	$\exp(\theta_{14})$	1.00	(b) (4)
$F$ 40mg/mL ~ FormulationStrength <sub>100mg/mL</sub>	$\exp(\theta_{15})$	0.762	(b) (4)
IIVvar CL ( $\omega_{CL}^2$ )	$\Omega_{1,1}$ ( $\eta_1$ )	0.195 (%CV=46.5)	(b) (4)
IIVcov CL, $V_2$ ( $\omega_{CL}\omega_{V_2}$ )	$\Omega_{2,1}$	-0.0601	(b) (4)
IIVvar $V_2$ ( $\omega_{V_2}^2$ )	$\Omega_{2,2}$ ( $\eta_2$ )	0.549 (%CV=85.5)	(b) (4)
IIVcov CL, $V_3$ ( $\omega_{CL}\omega_{V_3}$ )	$\Omega_{3,1}$	0.0344	(b) (4)
IIVcov $V_2$ , $V_3$ ( $\omega_{V_2}\omega_{V_3}$ )	$\Omega_{3,2}$	0.280	(b) (4)
IIVvar $V_3$ ( $\omega_{V_3}^2$ )	$\Omega_{3,3}$ ( $\eta_3$ )	0.168 (%CV=42.7)	(b) (4)
IIVcov CL, $k_a$ ( $\omega_{CL}\omega_{k_a}$ )	$\Omega_{4,1}$	-0.139	(b) (4)
IIVcov $V_2$ , $k_a$ ( $\omega_{V_2}\omega_{k_a}$ )	$\Omega_{4,2}$	0.0493	(b) (4)
IIVcov $V_3$ , $k_a$ ( $\omega_{V_3}\omega_{k_a}$ )	$\Omega_{4,3}$	-0.0585	(b) (4)
IIVvar $k_a$ ( $\omega_{k_a}^2$ )	$\Omega_{4,4}$ ( $\eta_4$ )	0.801 (%CV=111)	(b) (4)
Res <sub>additive</sub> ( $\sigma_{additive}$ )	$\Sigma_{1,1,additive}$ ( $\epsilon_1$ )	0.136 (SD=0.4)	(b) (4)

(Source: Applicants Response to FDA Information Request, 03 Feb 2015, Table 1)

**Figure 14. Comparison of Observed Values versus Population Predictions (top rows) and Observed Values versus Individual Predictions (bottom rows) between Pop-PK models with (right columns) and without (left columns) the Formulation Strength Covariate. The axes in the left panel are scaled to show all the data. The axes in the right panel are scaled to show the cluster of the majority of PK values.**



Blue straight line is the line of unity; the dashed red line is a trend line.

(Source: Applicants Response to FDA Information Request, 03 Feb 2015, Figure 2)

**Reviewer’s Comments:**

*The applicant’s inclusion of formulation strength appears reasonable to capture its effect on bioavailability of asfotase alfa. It is reassuring that the other parameter estimates were unchanged in the model.*

**3.4 Exposure Response Analyses:**

The applicant’s exposure response analyses covered a range of endpoints that were collected across the asfotase alfa clinical trials. Figure 4 illustrates the various levels of endpoints that were available for analysis.

**3.4.1 Overall Survival: Infantile Onset Population**

Overall survival was the Applicant’s pre-specified primary efficacy endpoint for the infantile-onset population.

“The pop-PK model was translated from the analytical algebraic prediction routine to a system of differential equations to allow for numerical integration of continuous PK histories and the derivation of average concentration since first dose, calculated as the cumulative area under the concentration-time curve (AUCcumulative)/time since first dose (Cavg, over the entire study) for

each individual. These individual Cavg values were analyzed as a continuous covariate along with “Year of Diagnosis” as predictors in a Cox proportional hazard model.

“For each unit increase in (U/L) of asfotase alfa PK activity the hazard for overall survival was scaled proportionally and statistically significantly ( $p = 0.0041$ ) by a coefficient of  $-0.001$ . The Hazard Ratio for one unit increase in Cavg (95% CI) was  $0.999$  ( $0.998-1.000$ ) Based on this analysis for overall survival, exposures associated with  $6 \text{ mg/kg}$  weekly (given either as  $2 \text{ mg/kg}$  three times weekly or  $1 \text{ mg/kg}$  six times weekly;  $1430$  to  $2930 \text{ U/L}$ ) is expected to improve the odds for overall survival significantly.

“It is important to note the limitations of this survival analysis. The relationship of increased survival with increased asfotase alfa PK activity (Cavg) may be confounded by the fact that more events took place in the Natural History arm, which was assigned a  $\text{Cavg}=0 \text{ U/L}$ . A survival analysis using data only from patients treated with drug was not performed as a Cox proportional hazard model with only four events (based on the data cut used for this analysis) would not provide meaningful information on the effect of Cavg. As a result it was not possible to assess whether there is/are a subpopulation(s) of patients who did not respond and may benefit from further dose modification or optimization. In summary, the Cox proportional hazard model analysis based on exposure offers limited additional benefit over the survival analysis that is based only on treatment status.”

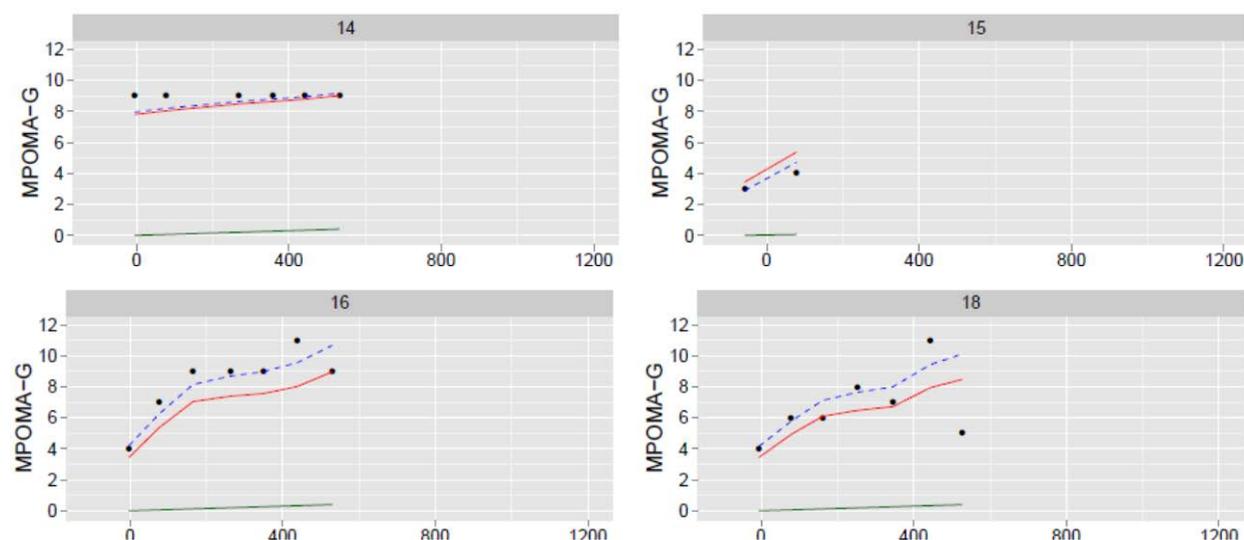
(Source: Applicant’s Summary of Clinical Pharmacology, Section 3.2.2.1.5)

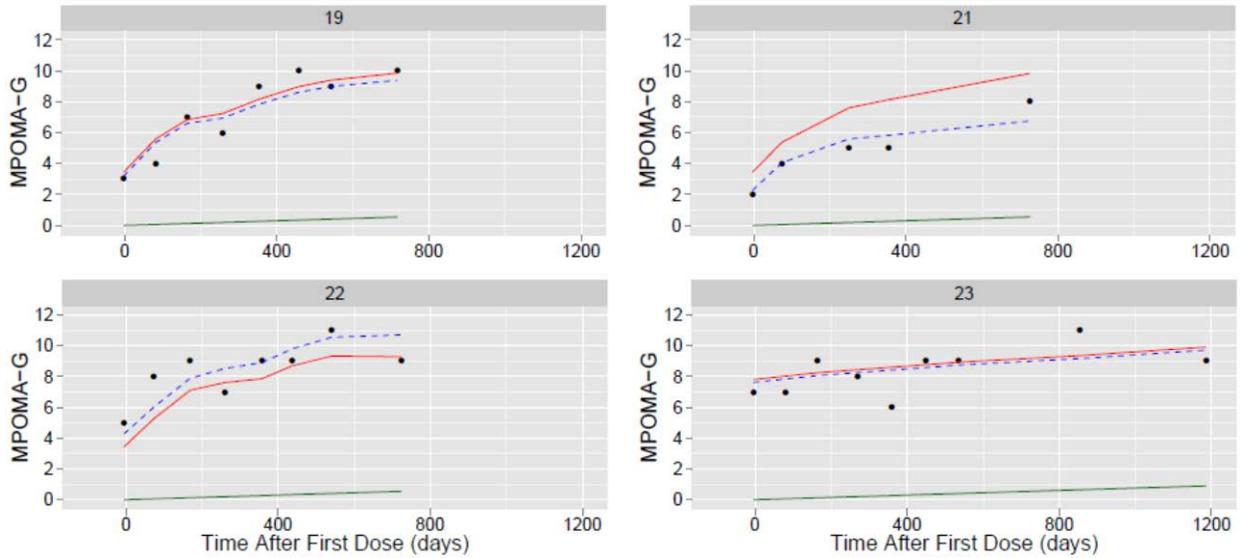
### 3.4.2 MPOMA-G Gait Analysis: Juvenile Onset Population

MPOMA-G Score was the primary efficacy endpoint for the juvenile-onset population.

Figure 14 depicts the exposure-response model fits for each of the eight individuals on asfotase alfa treatment that this data was collected for. The  $\text{EC}_{50}$  parameter in this model was  $1620 \text{ U/L}$  suggesting that concentrations above this would be necessary for the most treatment response.

**Figure 15. Individual model fits for MPOMA-G Score. Closed circles depict the observed values. The red line is the population prediction. The blue line is the individual estimation.**

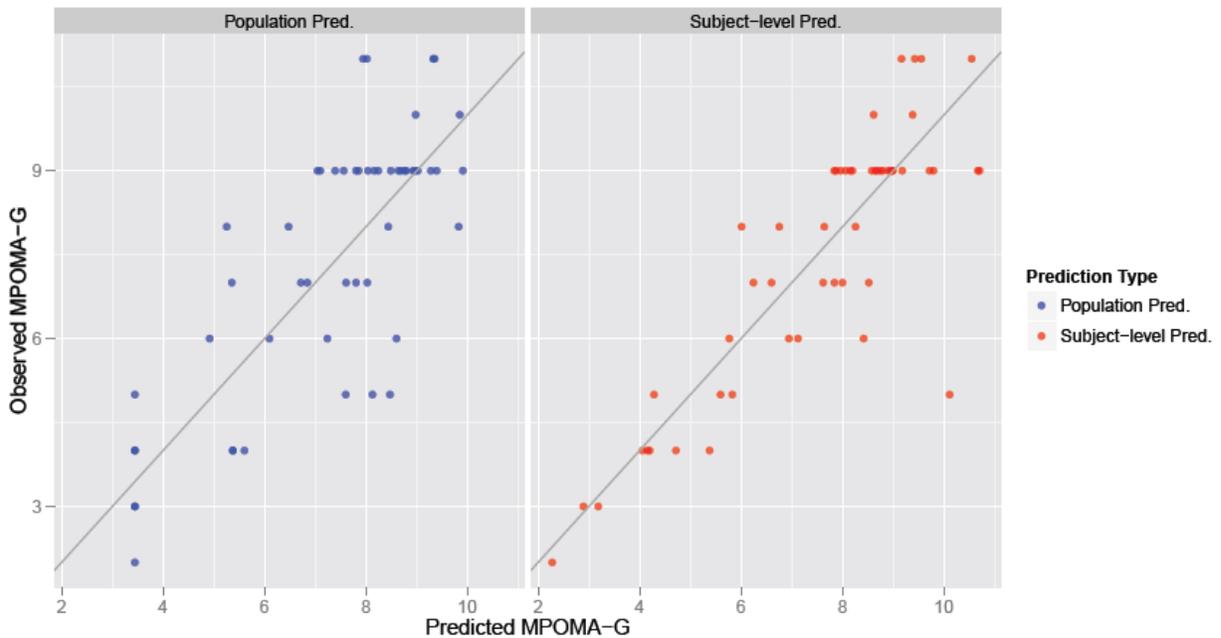




(Source: Applicant’s Population PK Report, December 2014, Figure 4)

Exposure-MPOMA-G model goodness of fit plots are shown in Figure 15 and suggest that the model is capturing the central tendency of the data.

**Figure 16. Observed versus population and individual predicted values for the final PK/MPOMA-G model. Values are indicated by the closed circles. The solid line is the line of identity.**



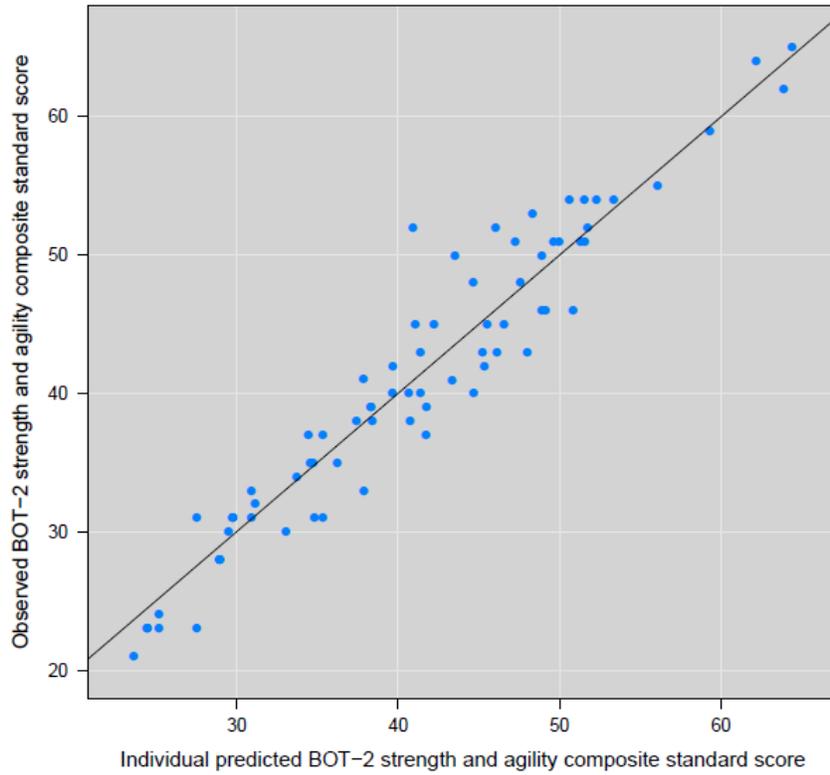
(Source: Applicant’s Population PK Report, December 2014, Figure 5)

### 3.4.3 Strength & Agility Scores and 6-Minute Walk Distance

Plots for the applicant’s final PK/PD for BOT-2 score and the 6-minute walk distance models are shown in Figure 2. These plots indicate that the plateau of maximal response is reached at or near 2000 U/L of asfotase alfa plasma concentration for both endpoints.

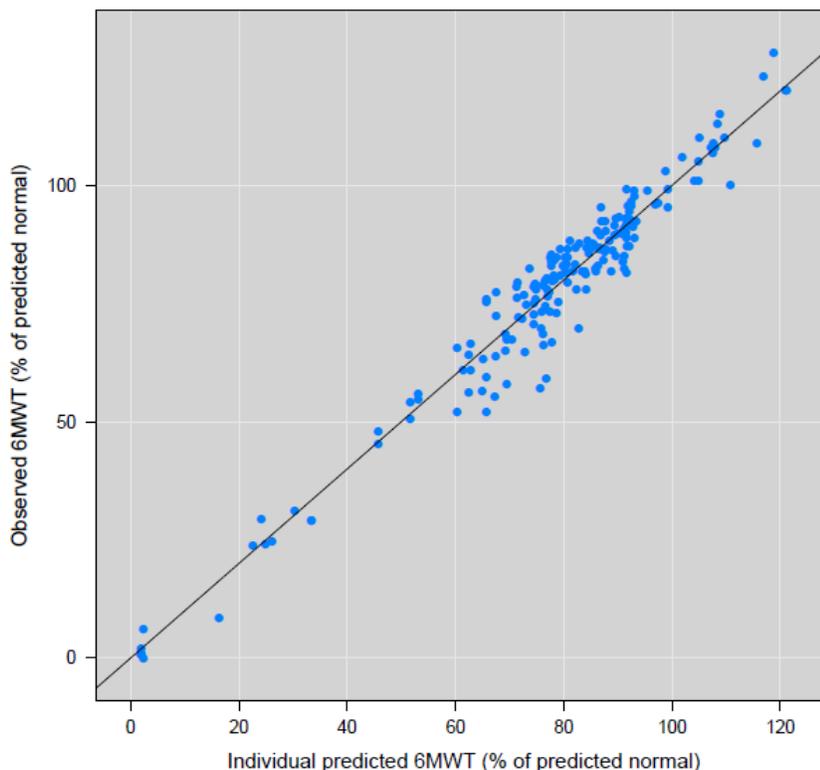
Diagnostic goodness of fit plots are shown in Figure 16 and Figure 17 and suggest that the model is capturing the central tendency of the data.

**Figure 17. BOT-2 Strength and Agility Composite Standard Score: Observed vs. Individual Predictions for the Final Population PK/BOT-2 Model. Values are indicated by closed circles. The solid line is the line of identity.**



(Source: Applicant's Population PK Report, Figure 129)

**Figure 18. 6-Minute Walk Test (% of predicted normal): Observed vs. individual predicted for the final PK/6MWT model. Values are the closed circles. The solid line is the line of identity.**



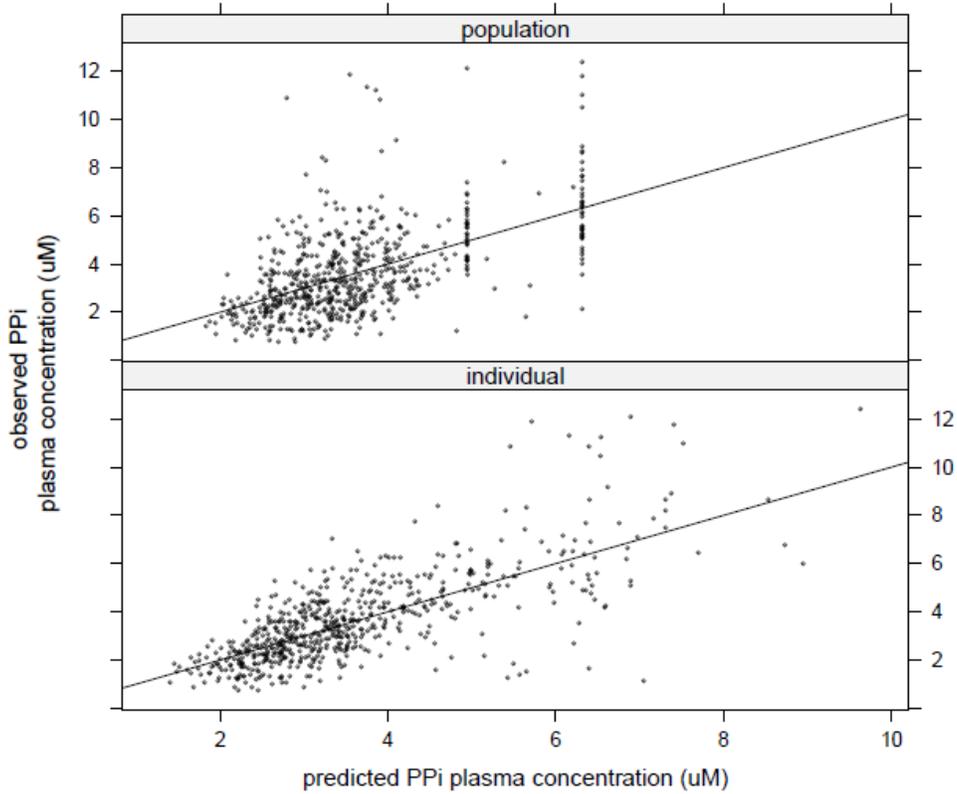
(Source: Applicant's Population PK Report, Figure 129)

### **3.4.4 Tissue Non-Specific Alkaline Phosphatase Substrates PPi and PLP**

Plots for the applicant's final PK/PD model are shown in Figure 3. These plots also indicate that the plateau of maximal response is reached at or near 2000 U/L of asfotase alfa plasma concentration for both endpoints.

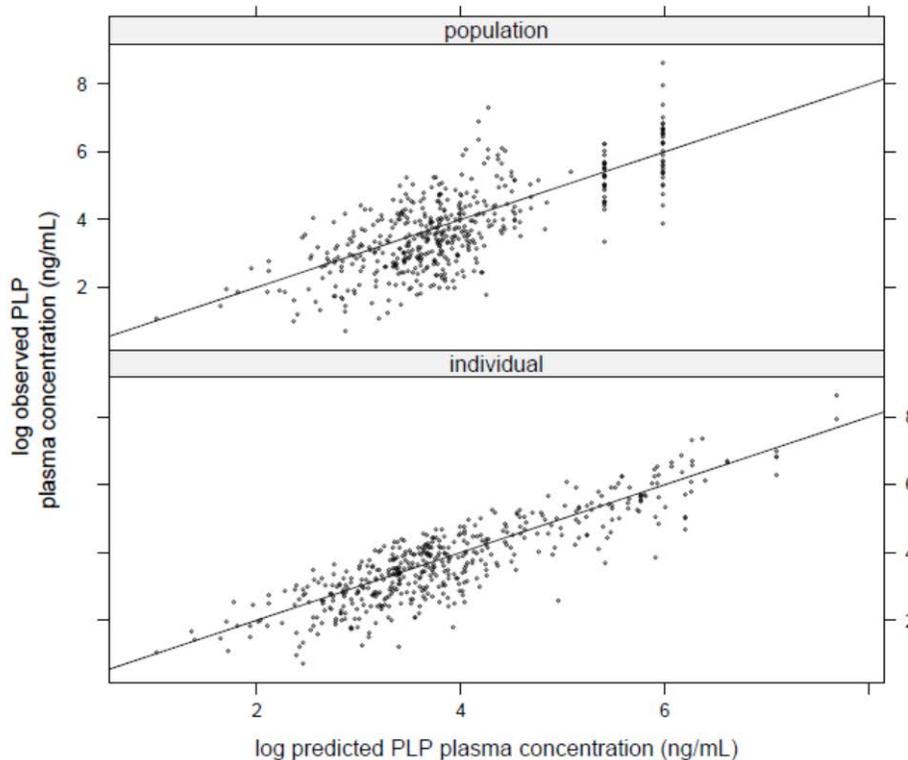
Diagnostic plots for goodness of fit are shown in Figure 18 and Figure 19 and suggest that the model is capturing the central tendency of the data.

**Figure 19. Observed versus Individual and Population Predicted Values for the Final PK/PPi Model. Observed PPi (uM) are plotted vs. population and individual predictions for the final population PD model. Values are indicated by open circles. The line of identity is included for reference.**



(Source: Applicant's Population PK Report, Figure 66)

**Figure 20. Observed versus Log Population and Individual Predicted Values for the final PK/PLP model. Values are indicated by open circles. The solid line is the line of identity.**



(Source: Applicant's Population PK Report, Figure 76)

**Reviewer's Comments:**

*The applicant's exposure response relationships appear reasonable to characterize the range of responses at different asfotase alfa exposures and identify that exposures at or above 2000 U/L would be ideal for dosing from an efficacy perspective.*

**4 REVIEWER'S ANALYSIS**

**4.1 Introduction**

The applicant's population PK analysis suggests two covariates that influence asfotase alfa exposure significantly, (b) (4) and body weight. The aim of the reviewer's analysis is to ascertain whether these covariate effects are accurate. Additionally, exposure-response for growth was evaluated to support the review team's assessment of clinical benefit of asfotase alfa.

**4.2 Objectives**

Analysis objectives are:

1. Determine if the effect (b) (4) on PK is accurate
2. Determine if patients with lower body weight should receive a higher dose than proposed.
3. Determine if an exposure-response relationship exists for the growth as indicated by z-score for height.

## 4.3 Methods

### 4.3.1 Data Sets

Data sets used are summarized in Table 4.

**Table 4. Analysis Data Sets**

Study Number	Name	Link to EDR
	mPOMA-G PKPD Analysis	<a href="\\cdsesub1\evsprod\BLA125513\0006\m5\datasets\pk-report-dec-2014">\\cdsesub1\evsprod\BLA125513\0006\m5\datasets\pk-report-dec-2014</a>
	Pop PK and other endpoint PKPD Analyses	<a href="\\cdsesub1\evsprod\BLA125513\0006\m5\datasets\pk-report-aug-2014">\\cdsesub1\evsprod\BLA125513\0006\m5\datasets\pk-report-aug-2014</a>

### 4.3.2 Software

The statistical software R (version 2.15, <http://www.r-project.org/>) was utilized for all plots and figures. NONMEM (Version 7.3) was used for running the applicant's population PK models.

### 4.3.3 Models

A population PK model approach was utilized to evaluate [REDACTED] (b) (4) when not included in the full model.

Often in population PK model diagnostics, the plots of eta versus the covariate of interest, can help inform the need for or the model fit of that covariate. If the covariate is not included in the model, trends in this type of plot indicate that it may be useful to explain between subject variability by including this covariate in the model. If the covariate is already included in the model, the distribution of etas should be centered around zero over the range of the covariate to ensure proper model characterization of the effect.

In the case of [REDACTED] (b) (4) as a covariate on Asfotase Alfa clearance, when the effect of [REDACTED] (b) (4) is excluded from the model, the single eta on clearance is insufficient to assess the need for this covariate. [REDACTED] (b) (4) changed between each batch that each individual received over time. Thus, when removing [REDACTED] (b) (4) as a covariate from the model, the eta for CL was no longer controlled by the fixed effect estimate of clearance at each occasion. And without evaluating an independent eta at each occasion of [REDACTED] (b) (4) for each individual, eta no longer can capture the need for this covariate.

To evaluate the magnitude of [REDACTED] (b) (4) and its relevance in the population PK model, the applicant's full model was rerun with the effect of [REDACTED] (b) (4) removed from the model and new eta values incorporated as between occasion [REDACTED] (b) (4) for each occurrence of different [REDACTED] (b) (4). While conditional weighted residuals permit assessment of the need for the covariate independent of eta, the eta allows us to ascertain the magnitude of the covariate effect on clearance without incorporating it in the model. Results of this analysis are shown in Section 1.1.2.

## 4.4 Results

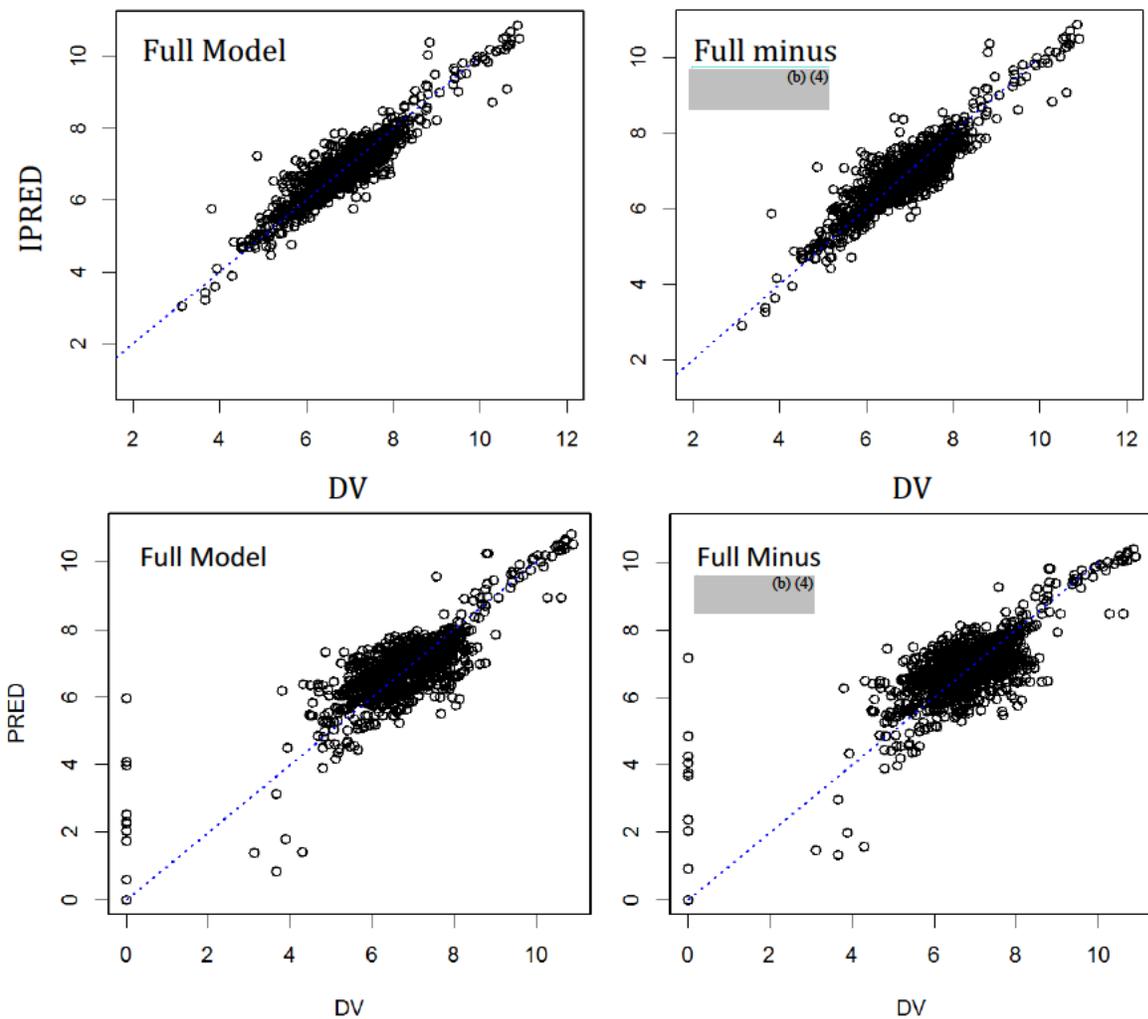
### 4.4.1 Should Patients the youngest patients receive a higher weight-based dose than the proposed 6 mg/kg/week dosing regimen?

No, the observed exposures in the youngest patients (< 6 months) were actually higher than majority of the population (Figure 1). While the applicant's model accounts for body weight as a covariate on the clearance of asfotase alfa, it does not account for the potential maturation of the clearance process in infants. Age was not detected as a covariate on clearance for the youngest patients. Figure 1 show the distribution of Cavg for the population by age as calculated from the individual post hoc Bayesian estimates of clearance (which includes the eta for each individual) and the average dose for each individual.

### 4.4.2 Is the assessment of the effect (b) (4) on Asfotase Alfa exposures by population PK robust?

Yes, the population PK analysis appears to appropriately include (b) (4) as a covariate on the clearance of asfotase alfa and the model estimate of the magnitude of that effect on clearance appears reasonable. Removal of (b) (4) as a covariate from the final permitted the comparison of the differences in the model with and without this covariate on clearance. The objective function increased by 260 suggesting this is a significant parameter based on a chi-squared test. There was improvement noted in the individual population PK predictions compared with the observations (Figure 20).

**Figure 21. Individual PK Predictions (top row) and Population PK Predictions (bottom row) versus Observed Values (DV) to Evaluate the Goodness of Fit Before (right column) and after (left column) the Inclusion of  $\text{[REDACTED]}^{(b)(4)}$  as a covariate on Asfotase Alfa Clearance.**



Additionally, conditional weighted residuals,  $\text{[REDACTED]}^{(b)(4)}$  dependent etas on CL, and non-compartmental Cavg values versus  $\text{[REDACTED]}^{(b)(4)}$  content suggest that it is reasonable to include  $\text{[REDACTED]}^{(b)(4)}$  as a covariate in the population PK model (Figure 21, Figure 22, and Figure 23).

Figure 22. Conditional Weighted Residuals versus <sup>(b) (4)</sup> Content for the Full Model with the Exclusion of <sup>(b) (4)</sup> Content (top panel) and the Full Model including <sup>(b) (4)</sup> as a covariate (bottom panel). Open circles depict CWRES and the blue line is a linear regression of the data.

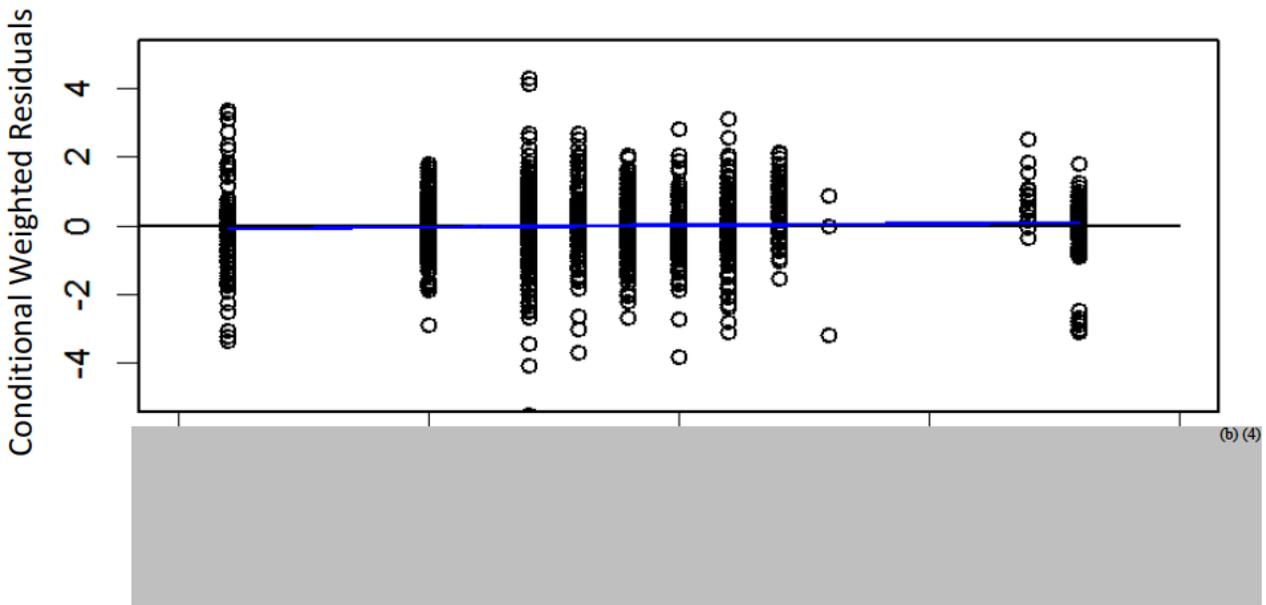
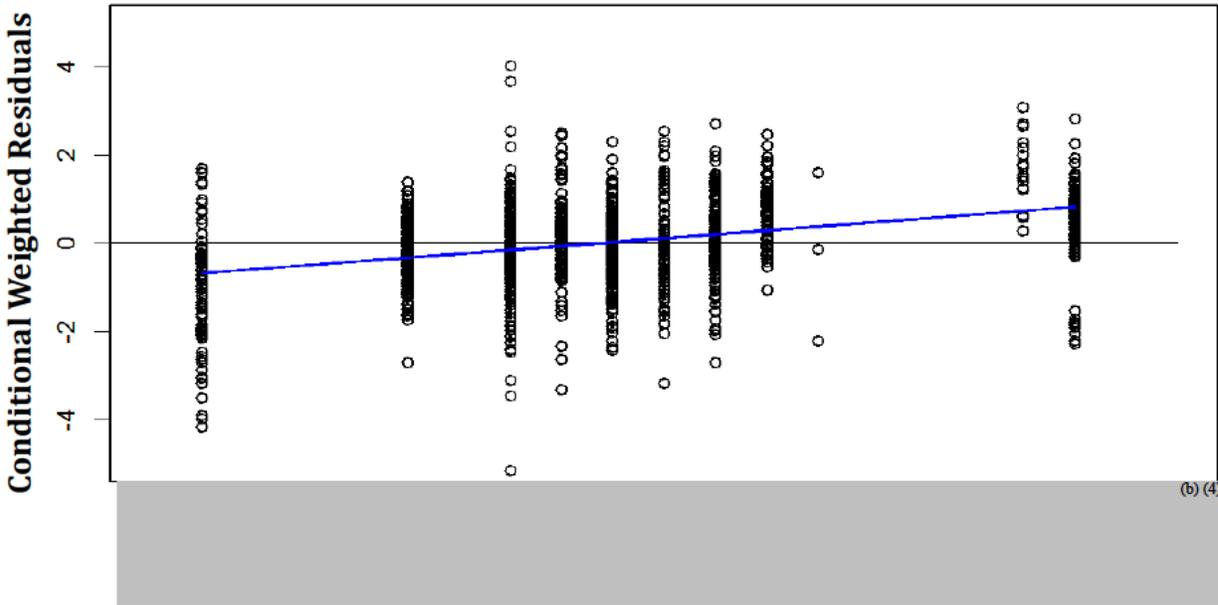
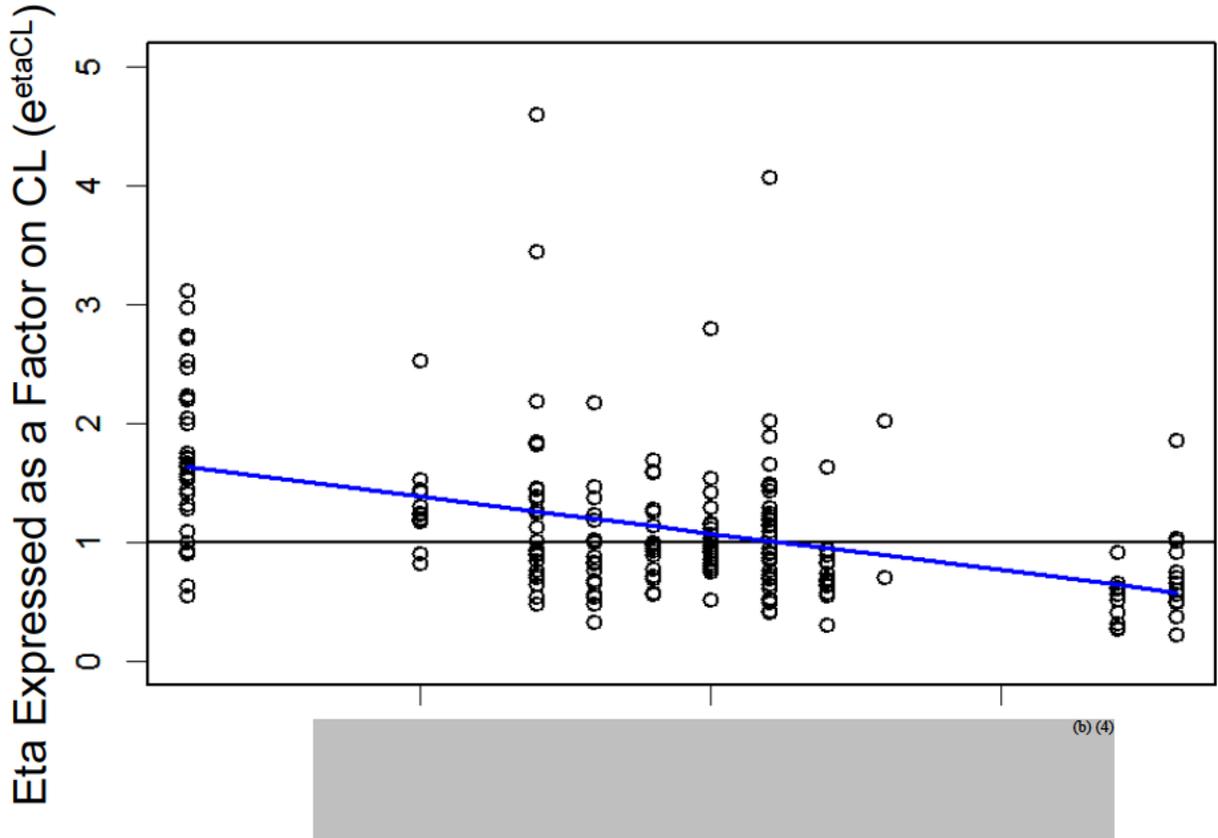
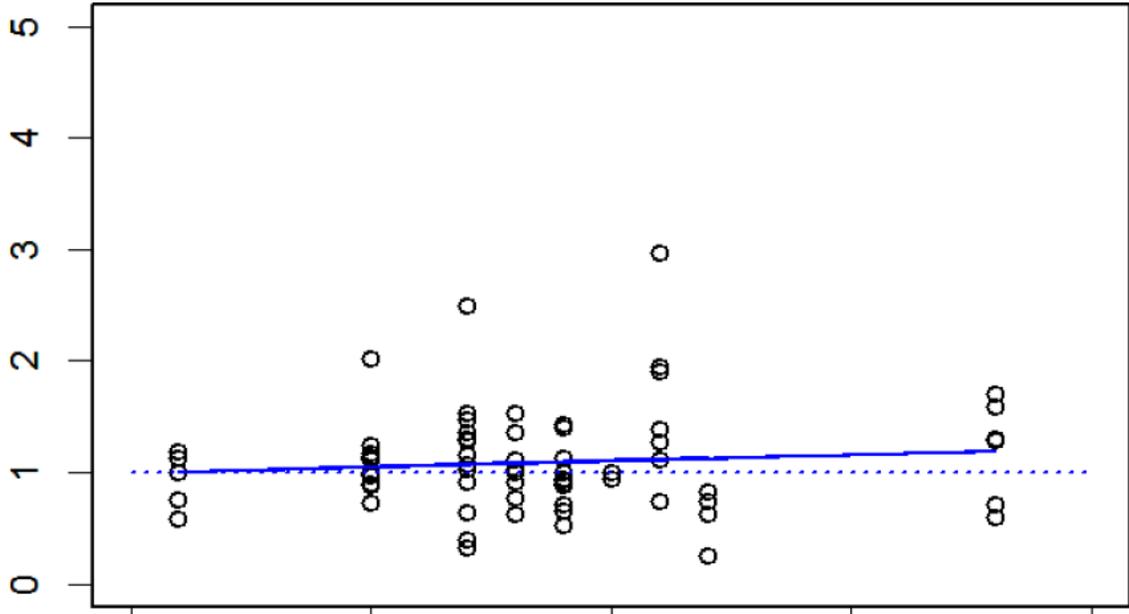


Figure 23. The effect of (b) (4) on Asfotase Alfa clearance for the model where (b) (4) is not a covariate (top panel) and for the full model including (b) (4) as a covariate (bottom panel). Eta is expressed as the factor on CL to indicate the magnitude of change on clearance between the individual post-hoc estimate of clearance and the model prediction for that individual. Open circles depict observations and the blue line is a linear regression of the data.

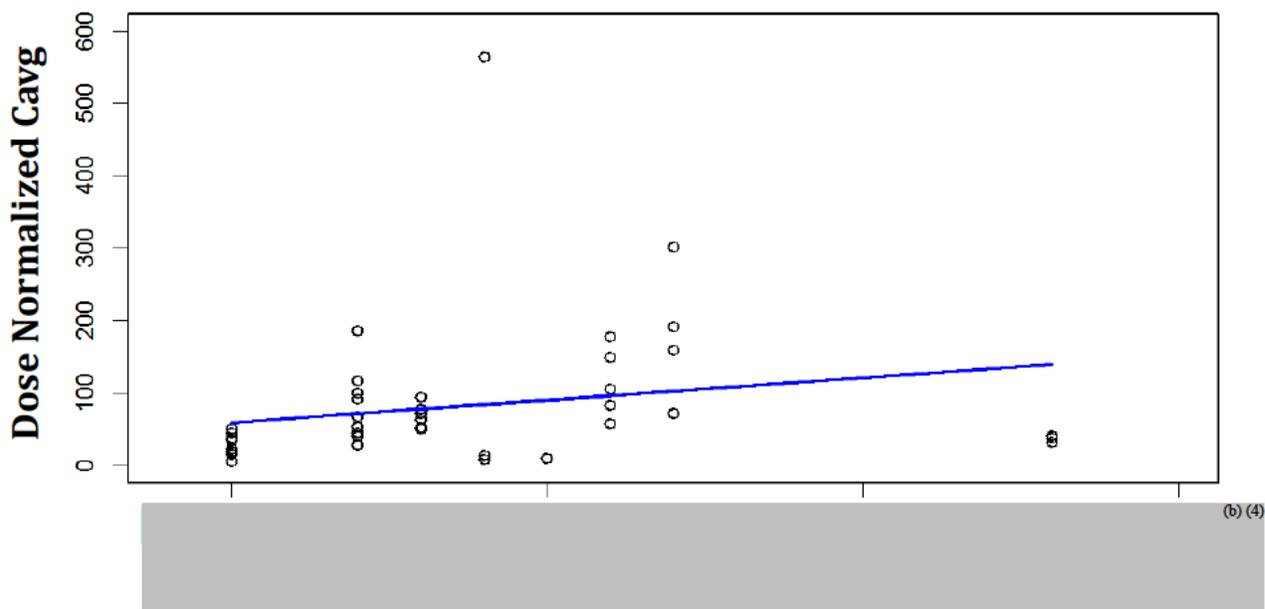


Eta Expressed as a Factor on CL ( $e^{\text{eta}_{\text{CL}}}$ )



(b) (4)

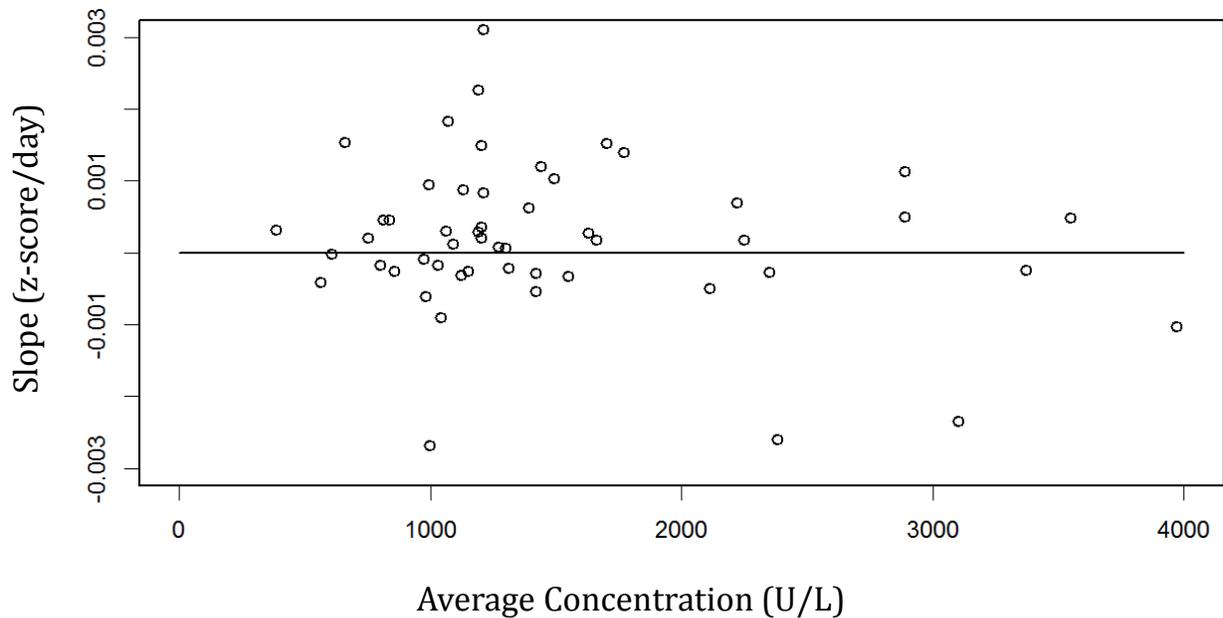
Figure 24. Dose Normalized Cavg versus (b) (4) Content. Dose Normalized Cavg was calculated from the PK samples during the first five days after the first dose in 45 subjects in the Asfotase Alfa population PK dataset from patients who had similar PK sampling during this time frame. Open circles depict observations and the blue line is a linear regression of the data.



#### 4.4.3 Exposure-Response for Growth

Exposure-response analysis for growth as indicated by Z-score for height was performed. The exposure metric chosen was the Cavg for each individual over the course of the study. The slope of Z-score for each individual was the dependent variable. Each individual's slope was calculated based on a linear mixed effects analysis that assumed inter-patient variability in both slope and intercept were present. Results from this time-averaged analysis suggest that there is no correlation between average asfotase alfa concentration and the overall growth rate of the patient relative to the normal population (Figure 24).

**Figure 25. Exposure-Response analysis for growth rate suggests there is no apparent correlation between individual Cavg and the slope of Z-score for height. The open circles depict the slope of the z-score over the duration of treatment for each individual and the solid line is a reference line at zero.**



## 5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location in \\cdsnas\pharmacometrics\
PopPK.R	R graphics for all Pop PK plots and the exposure response for growth analysis	..\Reviews\PM Review Archive\2015\Asfotase_Alfa_BLA125513_JCE\PPK Analyses
Asfotase.mmd	Berkeley Madonna Script for the Applicant's Models	..\Reviews\PM Review Archive\2015\Asfotase_Alfa_BLA125513_JCE\ER Analyses

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