Report on the Deliberation Results

June 9, 2015

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

Ministry of Health, Labour and Welfare

[Brand name]   Strensiq Subcutaneous Injection 12 mg/0.3 mL,

Strensiq Subcutaneous Injection 18 mg/0.45 mL,

Strensiq Subcutaneous Injection 28 mg/0.7 mL,

Strensiq Subcutaneous Injection 40 mg/1 mL,

Strensiq Subcutaneous Injection 80 mg/0.8 mL

[Non-proprietary name]   Asfotase Alfa (Genetical Recombination) (JAN*)

[Applicant]   Alexion Pharma G.K.

[Date of application]   October 15, 2014

[Results of deliberation]

In the meeting held on June 5, 2015, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The re-examination period is 10 years, the drug substance and the drug product are both classified as a powerful drug, and the product is classified as a biological product.

[Conditions for approval]

The applicant is required to:

• Develop a risk management plan and implement it appropriately.

• Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period to obtain characteristics of the patients because of the very limited number of Japanese patients included in the clinical studies, and collect data on the safety and efficacy of the product as soon as possible, so that appropriate measures can be taken to ensure its proper use.

Japanese Accepted Name (modified INN)

<div align="center">**Review Report**</div>

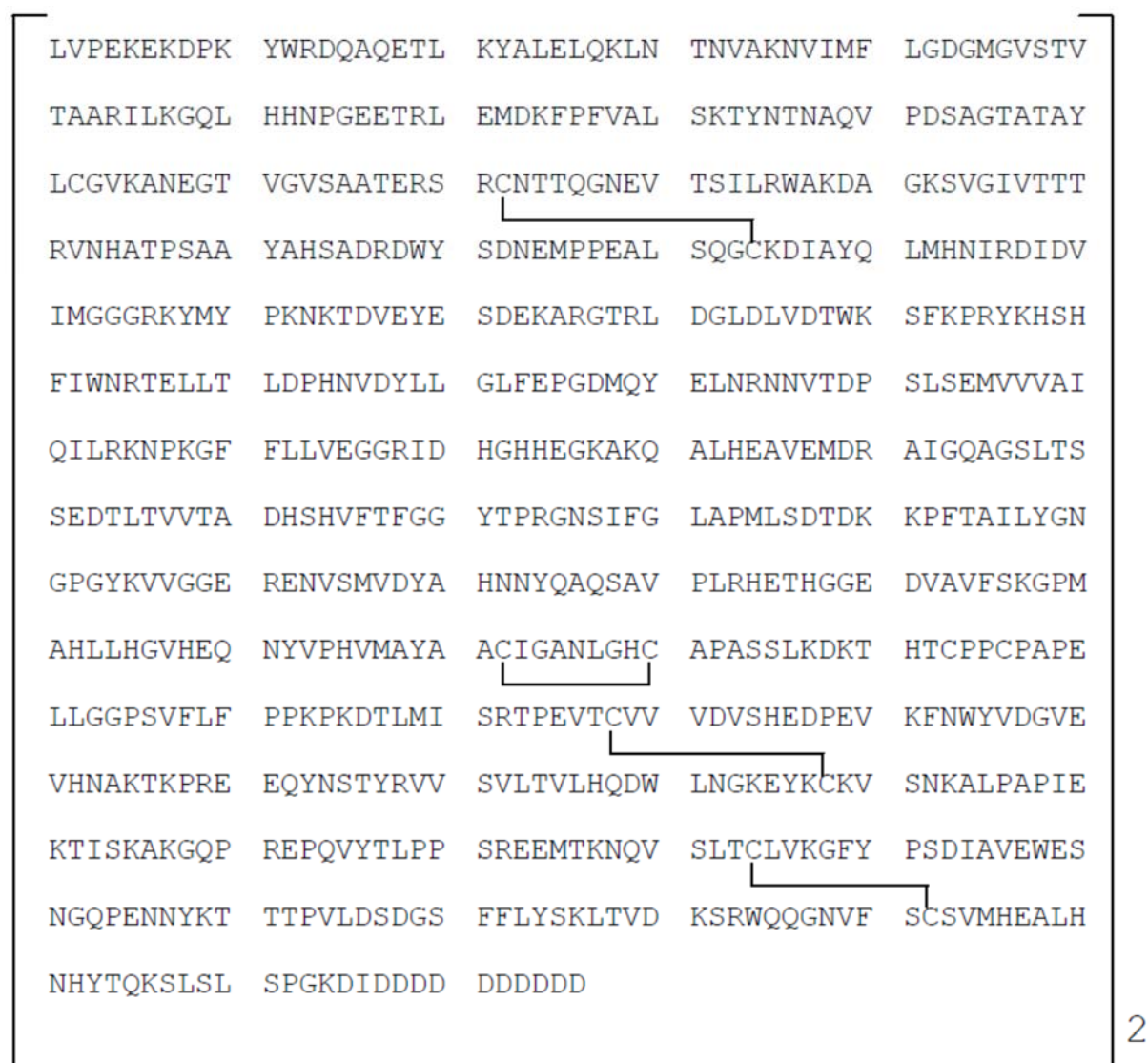<div align="right">May 18, 2015

Pharmaceuticals and Medical Devices Agency</div>

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Strensiq Subcutaneous Injection 12 mg/0.3 mL,
	Strensiq Subcutaneous Injection 18 mg/0.45 mL,
	Strensiq Subcutaneous Injection 28 mg/0.7 mL,
	Strensiq Subcutaneous Injection 40 mg/1 mL,
	Strensiq Subcutaneous Injection 80 mg/0.8 mL
[Non-proprietary name]	Asfotase Alfa (Genetical Recombination)
[Applicant]	Alexion Pharma G.K.
[Date of application]	October 15, 2014
[Dosage form/Strength]	Injection: Each vial (0.3 mL, 0.45 mL, 0.7 mL, 1 mL, or 0.8 mL of solution) contains 12 mg, 18 mg, 28 mg, 40 mg, or 80 mg, respectively, of Asfotase Alfa (Genetical Recombination).
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Definition]	Asfotase alfa is a recombinant fusion glycoprotein corresponding to a catalytic domain of human tissue non-specific alkaline phosphatase at positions 1-485, Fc domain of human Ig G1 at positions 488-714, and 10 residues of Asp are attached to the C-terminus. Asfotase alfa is produced in Chinese hamster ovary cells. Asfotase alfa is a glycoprotein (molecular weight: ca. 180,000) composed of 2 subunits consisting of 726 amino acid residues each.
[Chemical structure]	See Attachment
[Items warranting special mention]	Orphan drug (Drug Designation No. 346 of 2014 [*26 yaku*]; Notification No. 0821-1 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW, dated August 21, 2014)
[Reviewing office]	Office of New Drug I

Amino acid sequence and disulfide bridges:

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LVPEKEKDPK   YWRDQAQETL   KYALELQKLN   TNVAKNVIMF   LGDGMGVSTV

TAARILKGQL   HHNPGEETRL   EMDKFPFVAL   SKTYNTNAQV   PDSAGTATAY

LCGVKANEGT   VGVSAATERS   RCNTTQGNEV   TSILRWAKDA   GKSVGIVTTT

RVNHATPSAA   YAHSADRDWY   SDNEMPPEAL   SQGCKDIAYQ   LMHNIRDIDV

IMGGGRKYMY   PKNKTDVEYE   SDEKARGTRL   DGLDLVDTWK   SFKPRYKHSH

FIWNRTELLT   LDPHNVDYLL   GLFEPGDMQY   ELNRNNVTDP   SLSEMVVVAI

QILRKNPKGF   FLLVEGGRID   HGHHEGKAKQ   ALHEAVEMDR   AIGQAGSLTS

SEDTLTVVTA   DHSHVFTFGG   YTPRGNSIFG   LAPMLSDTDK   KPFTAILYGN

GPGYKVVGGE   RENVSMVDYA   HNNYQAQSAV   PLRHETHGGE   DVAVFSKGPM

AHLLHGVHEQ   NYVPHVMAYA   ACIGANLGHC   APASSLKDKT   HTCPPCPAPE

LLGGPSVFLF   PPKPKDTLMI   SRTPEVTCVV   VDVSHEDPEV   KFNWYVDGVE

VHNAKTKPRE   EQYNSTYRVV   SVLTVLHQDW   LNGKEYKCKV   SNKALPAPIE

KTISKAKGQP   REPQVYTLPP   SREEMTKNQV   SLTCLVKGFY   PSDIAVEWES

NGQPENNYKT   TTPVLDSDGS   FFLYSKLTVD   KSRWQQGNVF   SCSVMHEALH

NHYTQKSLSL   SPGKDIDDDD   DDDDDD
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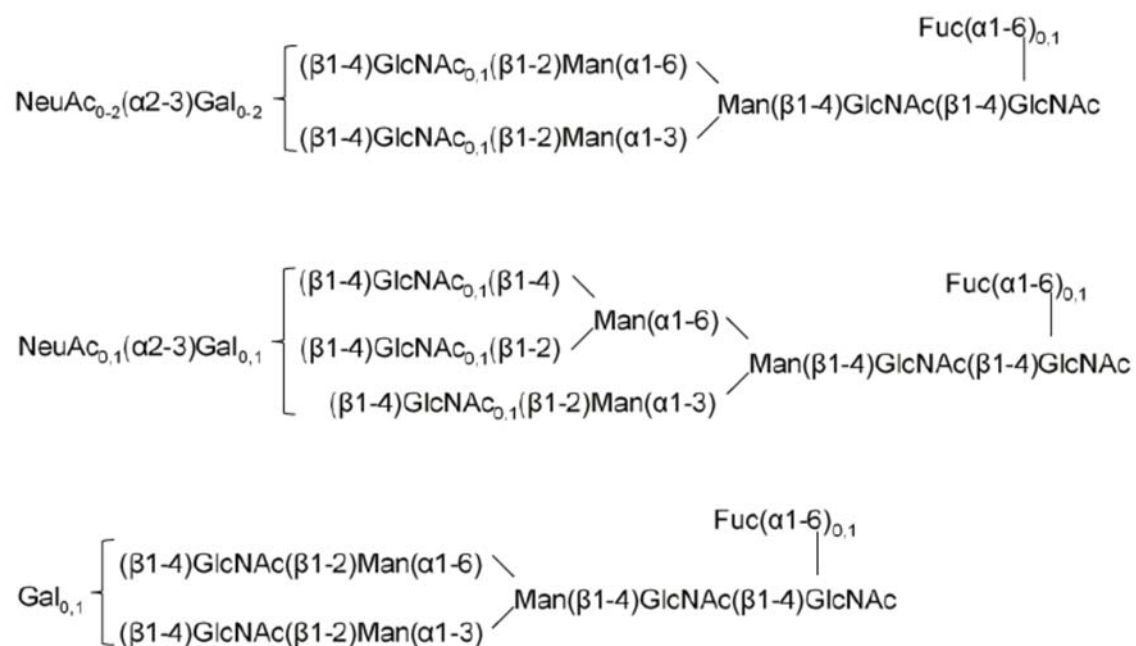
2

Disulfide bridges: C-C
Intersubunit disulfide bridges: C493-C493, C496-C496
Glycosylation sites: N123, N213, N254, N286, N413, N564

Putative structures of main carbohydrate chains:

$$\text{NeuAc}_{0-2}(\alpha 2\text{-}3)\text{Gal}_{0-2}\left[\begin{array}{l}(\beta 1\text{-}4)\text{GlcNAc}_{0,1}(\beta 1\text{-}2)\text{Man}(\alpha 1\text{-}6)\searrow\\(\beta 1\text{-}4)\text{GlcNAc}_{0,1}(\beta 1\text{-}2)\text{Man}(\alpha 1\text{-}3)\nearrow\end{array}\right]\begin{array}{c}\text{Fuc}(\alpha 1\text{-}6)_{0,1}\\|\\\text{Man}(\beta 1\text{-}4)\text{GlcNAc}(\beta 1\text{-}4)\text{GlcNAc}\end{array}$$

$$\text{NeuAc}_{0,1}(\alpha 2\text{-}3)\text{Gal}_{0,1}\left[\begin{array}{l}(\beta 1\text{-}4)\text{GlcNAc}_{0,1}(\beta 1\text{-}4)\searrow\\(\beta 1\text{-}4)\text{GlcNAc}_{0,1}(\beta 1\text{-}2)\nearrow\text{Man}(\alpha 1\text{-}6)\searrow\\(\beta 1\text{-}4)\text{GlcNAc}_{0,1}(\beta 1\text{-}2)\text{Man}(\alpha 1\text{-}3)\nearrow\end{array}\right]\begin{array}{c}\text{Fuc}(\alpha 1\text{-}6)_{0,1}\\|\\\text{Man}(\beta 1\text{-}4)\text{GlcNAc}(\beta 1\text{-}4)\text{GlcNAc}\end{array}$$

$$\text{Gal}_{0,1}\left[\begin{array}{l}(\beta 1\text{-}4)\text{GlcNAc}(\beta 1\text{-}2)\text{Man}(\alpha 1\text{-}6)\searrow\\(\beta 1\text{-}4)\text{GlcNAc}(\beta 1\text{-}2)\text{Man}(\alpha 1\text{-}3)\nearrow\end{array}\right]\begin{array}{c}\text{Fuc}(\alpha 1\text{-}6)_{0,1}\\|\\\text{Man}(\beta 1\text{-}4)\text{GlcNAc}(\beta 1\text{-}4)\text{GlcNAc}\end{array}$$

NeuAc, *N*-acetylneuraminic acid

GlcNAc, *N*-acetylglucosamine

Gal, galactose

Man, mannose

Fuc, fucose

Molecular formula (protein moiety)

Dimer: $C_{7108}H_{11008}N_{1968}O_{2206}S_{56}$ (molecular weight = 161,122.96)

Monomer: $C_{3554}H_{5506}N_{984}O_{1103}S_{28}$ (molecular weight = 80,563.50)

<center>**Review Results**</center>

<div align="right">May 18, 2015</div>

[Brand name]	Strensiq Subcutaneous Injection 12 mg/0.3 mL,
	Strensiq Subcutaneous Injection 18 mg/0.45 mL,
	Strensiq Subcutaneous Injection 28 mg/0.7 mL,
	Strensiq Subcutaneous Injection 40 mg/1 mL,
	Strensiq Subcutaneous Injection 80 mg/0.8 mL
[Non-proprietary name]	Asfotase Alfa (Genetical Recombination)
[Applicant]	Alexion Pharma G.K.
[Date of application]	October 15, 2014

[Results of review]

Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that the efficacy of the product in the treatment of hypophosphatasia has been demonstrated and its safety is acceptable in view of its observed benefits. PMDA considers that further review through post-marketing surveillance is needed to investigate the effects of injection-site reactions, injection-related reactions, serum calcium fluctuations, and antibody production on safety and efficacy, the safety of long-term use, and other issues.

As a result of its regulatory review, PMDA has concluded that the product may be approved for the indication and dosage and administration as shown below, with the following conditions.

[Indication]

Hypophosphatasia

[Dosage and administration]

The usual dosage of Asfotase Alfa (Genetical Recombination) is 1 mg/kg administered subcutaneously 6 times weekly or 2 mg/kg administered subcutaneously 3 times weekly. The dose should be reduced appropriately according to the patient's condition.

[Conditions for approval]

The applicant is required to:

• Develop a risk management plan and implement it appropriately.
• Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period to obtain characteristics of the patients because of the very limited number of Japanese patients included in the clinical studies, and collect data on the safety and efficacy of the product as soon as possible, so that appropriate measures can be taken to ensure its proper use.

<center>5</center>

Review Report (1)

March 27, 2015

[Brand name]	Strensiq Subcutaneous Injection 12 mg/0.3 mL,
	Strensiq Subcutaneous Injection 18 mg/0.45 mL,
	Strensiq Subcutaneous Injection 28 mg/0.7 mL,
	Strensiq Subcutaneous Injection 40 mg/1 mL,
	Strensiq Subcutaneous Injection 80 mg/0.8 mL
[Non-proprietary name]	Asfotase Alfa (Genetical Recombination)
[Applicant]	Alexion Pharma G.K.
[Date of application]	October 15, 2014
[Dosage form/Strength]	Injection: Each vial (0.3 mL, 0.45 mL, 0.7 mL, 1 mL, or 0.8 mL of solution) contains 12 mg, 18 mg, 28 mg, 40 mg, or 80 mg, respectively, of Asfotase Alfa (Genetical Recombination).
[Proposed indication]	Hypophosphatasia
[Proposed dosage and administration]	The usual dosage is 6 mg/kg/week of Asfotase Alfa (Genetical Recombination) administered subcutaneously. The dose is administered either at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly according to the patient's condition.

II. Summary of the Submitted Data and Outline of the Review by Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

Strensiq 12 mg/0.3 mL, 18 mg/0.45 mL, 28 mg/0.7 mL, 40 mg/1 mL, and 80 mg/0.8 mL for Subcutaneous Injections (hereinafter collectively referred to as "Strensiq") are injectable preparations containing Asfotase Alfa (Genetical Recombination) (hereinafter referred to as "asfotase alfa"), developed by Enobia Pharma Corp., as the active substance.

Hypophosphatasia is a genetic disorder caused by loss-of-function mutations in the gene encoding tissue non-specific alkaline phosphatase (TNSALP),[1] and the resulting increases in the concentrations of TNSALP substrates such as inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) impair bone mineralization, phosphate regulation, and calcium regulation, with main clinical signs consisting of progressive multiple organ dysfunction including deformity and destruction of bones, pain, marked muscle weakness, respiratory failure, convulsive seizures, renal impairment, and dental abnormalities. The common forms of hypophosphatasia are classified by age of onset into perinatal, infantile, childhood,

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[1] Whyte MP. *Genetics of Bone Biology and Skeletal Disease.* Thakker RV, et al eds. San Diego: Elsevier; 2013:337-60

and adult types. The major cause of death among non-adult patients is respiratory failure,[2] and the mortality is 50% to 100% in the most severe cases.[3]

The incidence of hypophosphatasia is estimated to be one in every 100,000 live births outside Japan,[4] and the number of patients with the disease is estimated to be 100 to 200 in Japan.[5]

In Japan, no drug product has been approved for the treatment of hypophosphatasia, and the following symptomatic treatment options are currently available[6]: mechanical ventilation by endotracheal intubation for spontaneous respiratory failure due to insufficient thoracic cage development in infancy; calcium-restricted diets for hypercalcemia due to impaired bone mineralization or diuretic administration to remove calcium; administration of vitamin B_6 preparations for convulsive seizures due to vitamin B_6 deficiency in the central nervous system; and surgical intervention for craniosynostosis due to mineralization of membranous bones.

Asfotase alfa is a fusion protein preparation consisting of the catalytic domain of human TNSALP, the fragment crystallizable (Fc) domain of human immunoglobulin (Ig) G1, and a deca-aspartate peptide. It replaces deficient TNSALP, which hydrolyses PPi, an inhibitor of bone mineralization, and the resulting inorganic phosphate (Pi) binds to calcium, thereby promoting deposition of hydroxyapatite crystals and bone mineralization, and inducing normal skeletal development.

Asfotase alfa is designated as an orphan drug (Drug Designation No. 346 of 2014 [*26 yaku*]) with the proposed indication for the treatment of hypophosphatasia.

Claiming that clinical studies including a global clinical study (ENB-010-10) have demonstrated the efficacy and safety of asfotase alfa in the treatment of hypophosphatasia, the applicant has filed a new drug application.

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[2] Chest deformity related to rickets has been reported to make it difficult for the thorax to maintain normal respiratory function; thereby leading to the necessity of providing ventilation support or to increased risk of fatal respiratory failure.

[3] Caswell AM, et al. *Crit Rev Clin Lab Sci*. 1991;28:175-232; Whyte MP. *Pediatric Bone, Biology and Disease*. Glorieux FH, et al eds. London, Academic Press, 2012;771-94; Greenberg CR, et al. *Genomics*. 1993;17:215-7

[4] Fraser D. *Am J Med*. 1957;22:730-46; Mornet E, et al. *Ann Hum Genet*. 2011;75:439-45

[5] Study Report for Fiscal Years 2009 to 2011 Research on Optimal Personalized Treatment of Hypophosphatasia, Health and Labour Sciences Research Grants, Research Project on Treatment of Intractable Diseases

[6] Caswell AM, et al. *Crit Rev Clin Lab Sci*. 1991;28:175-232

2. Data relating to quality

2.A   Summary of the submitted data

2.A.(1) Drug substance

2.A.(1).1) Preparation and control of cell substrates

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██████████████████████████████████ The expression construct containing the gene of interest was transfected into Chinese hamster ovary (CHO) cell lines, from which a clone optimized in terms of asfotase alfa productivity, maximum cell density, and doubling time was selected and used as the source for establishing the master cell bank (MCB) and working cell bank (WCB).

Genetic stability during manufacture was demonstrated in MCB, WCB, and cells at the limit of *in vitro* cell age used for production (CAL) by the following characterizations: Southern blotting analysis, gene copy count, Northern blotting analysis, and cDNA sequencing, or viability. The purity of MCB, WCB, and CAL was also assessed by the following assays: sterility assay, mycoplasma testing (culture and DNA staining), mouse minute virus (MMV) detection assay, hamster antibody production assay, co-culture with retrovirus, transmission electron microscopy, high-sensitivity reverse transcriptase assay, adventitious virus assay (*in vitro* and *in vivo*), *in vitro* bovine virus assay, *in vitro* porcine virus assay, and extended S^+L^- assay, or extended XC plaque assay. The results showed the absence of adventitious viruses or non-viral infective agents within the scope of testing, except for endogenous retrovirus and retrovirus-like particles common to murine cell lines.

The MCB and WCB are stored frozen in liquid nitrogen. Neither MCB nor WCB will be replaced.

2.A.(1).2) Manufacturing process

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██ The drug substance obtained is stored in a multi-layer container[7] at ██ to ██ °C.

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██████████████████

Process validation of the commercial-scale manufacturing process for the drug substance has been conducted.

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[7] ██

2.A.(1).3) Safety evaluation of adventitious agent

No biological ingredients other than CHO cells, the host cells, are used in the manufacturing process for the drug substance.

Purity tests have been performed for the MCB, WCB, and CAL [see "2.A.(1).1) Preparation and control of cell substrates"]. The culture media is assessed before harvest by the following in-process control tests: bioburden assay, mycoplasma testing, *in vitro* virus assay, and MMV assay.

Viral clearance tests using model viruses demonstrated that the manufacturing process has consistent viral clearance capacity (Table 1).

Table 1. Results of viral clearance tests

Manufacturing process	Virus reduction factor ($\log_{10}$)			
	Xenotropic murine leukemia retrovirus	Pseudorabies virus	Reovirus type 3	Mouse minute virus
Virus inactivation	█	█	█	█
█████	█	█	█	█
████	█	█	██	█
Virus filtration	█	█	█	█
Total virus reduction factor	≥18.69	≥10.84	≥16.61	≥9.37

2.A.(1).4) Manufacturing process development (comparability)

Major changes in the manufacturing process during the development of the drug substance are shown below (the respective manufacturing methods are as follows: Method █ L for pilot scale batches, and Methods A1, A2, A3, A4, A5, A6, and A7 [proposed manufacturing method]). Clinical studies were conducted using the drug substance produced by Method A1 and subsequent methods.

- From Method █ L for pilot scale batches to Method A1, a change in manufacturing scale
- From Method A1 to Method A2, ████████████████████
- From Method A2 to Method A3, ███████████
- From Method A3 to Method A4, ████████████████
- From Method A4 to Method A5, ██████████████
- From Method A5 to Method A6, █████████████
- From Method A6 to Method A7, █████████████████

For these changes in the manufacturing process, comparability studies on quality attributes were conducted and the comparability of the drug substances before and after the changes in the manufacturing process was confirmed.

2.A.(1).5) Characterization

(a) Structure

- The primary structure of the drug substance has been elucidated by amino acid composition analysis, N-terminal amino acid sequencing, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS), electrospray ionization-time of flight mass spectrometry (ESI-TOF-MS), and tryptic peptide mapping.

- The higher-order structure of the drug substance has been elucidated by circular dichroism spectroscopy in the far- and near-ultraviolet regions, non-reduced alkylated tryptic peptide mapping, and sulfhydryl group analysis.

- The metal contents were determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES).

- Phosphorylation sites and the ratio of phosphorylation have been determined by reduced alkylated tryptic peptide mapping and tandem mass spectrometry.

- Glycosylation sites and sugar chain structures were determined by analysis of free sugar chains by MALDI-TOF-MS, 2-aminobenzamide-labeled free sugar chain analysis, reduced alkylated tryptic peptide mapping, and measurement of sialic acid content.

(b) Physicochemical properties

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████████████████████████████████

- ██
████████████████████████████

(c) Biological properties

- The alkaline phosphatase activity was determined by enzyme activity assay using *p*-nitrophenyl phosphate (pNPP) as substrate.

- The hydroxyapatite (HA) binding ratio was determined by HA binding assay [see "3.(i).A.(1).1) (a) Binding to hydroxyapatite (4.2.1.1.9)"].

- The Michaelis constant and reaction rate constant were determined by hydrolysis assay using inorganic pyrophosphate (PPi) as substrate.

- The results of surface plasmon resonance assay confirmed that the drug substance has binding activity for the Fc receptor.

- Complement activation assay was performed using human serum to confirm that asfotase alfa has no effector function. The assay detected no activation of complement cascade.

(d) Product-related substances, product-related impurities

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████████████████████████████████

(e) Process-related impurities

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██ These impurities have been

demonstrated to be sufficiently removed through the manufacturing process.

2.A.(1).6) Control of drug substance

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2.A.(1).7) Stability of drug substance

A summary of the main stability studies of the drug substance is as shown in Table 2.

Table 2. Summary of the main stability studies of the drug substance

	Number of batches	Storage condition	Storage period	Storage container
Long-term	5 batches[a]	5 ± 3°C	12 months[b]	Multi-layer container
Accelerated	3 batches[a]	25 ± 2°C	12 months	

a) Method A7; b) The stability study is ongoing.

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Based on the results of the above studies and a photostabiliy study of the 80 mg/0.8 mL formulation, a shelf life of 12 months has been proposed for the drug substance when stored at 2 to 8°C in a multi-layer container protected from light. The long-term testing will be continued for up to 24 months.

2.A.(2) Drug product
2.A.(2).1) Description and composition of the drug product, and formulation design

The drug product is an injectable preparation available in vials each containing 12 mg/0.3 mL, 18 mg/0.45 mL, 28 mg/0.7 mL, 40 mg/1 mL, or 80 mg/0.8 mL of asfotase alfa. Each drug product contains the following excipients: sodium chloride, sodium phosphate dibasic heptahydrate, and sodium dihydrogen phosphate monohydrate. The primary package is a glass vial (2 mL) with a rubber stopper, and the secondary package is a paper box.

2.A.(2).2) Manufacturing process

The manufacturing process of the drug product comprises drug solution preparation, sterile filtration, filling and capping, testing, labeling and packaging, and storage and testing steps. ███

Process validation of the commercial-scale manufacturing process for the drug product has been conducted.

2.A.(2).3) Manufacturing process development

The manufacturing site was changed during the development of the drug product. Additionally, only 100 mg/mL was selected for the strength of the drug substance, thus, a step for dilution of the drug substance was added to the manufacturing process of the 40 mg/mL formulation. The results obtained in studies on quality attributes have indicated the comparability of the drug products produced before and after the change of the manufacturing site.

2.A.(2).4) Control of drug product

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2.A.(2).5) Stability of drug product

A summary of the main stability studies of the drug product was as shown in Table 3. Stability studies were conducted using formulations manufactured from drug substances that were produced in accordance with Methods A2 to A7.

Table 3. Summary of the main stability studies of the drug product

	Specification	Strength	Storage condition	6 months	12 months	18 months	24 months	30 months	36 months	Storage container
Long-term	12 mg/0.3 mL	40 mg/mL	5 ± 3°C Upright and inverted			2	1			Glass vial
	18 mg/0.45 mL				1					
	28 mg/0.7 mL						1	1a)	1	
	40 mg/1 mL			1		1	1			
	80 mg/0.8 mL	100 mg/mL			1	1	2			

	Specification	Strength	Storage condition	6 months		12 months		Storage container
Accelerated	12 mg/0.3 mL	40 mg/mL	25 ± 2°C Upright and inverted			3		Glass vial
	18 mg/0.45 mL					1		
	28 mg/0.7 mL			2a)		1		
	40 mg/1 mL			1		2		
	80 mg/0.8 mL	100 mg/mL				4		

	Specification	Strength	Storage condition		Storage container
Photostability	12 mg/0.3 mL	40 mg/mL	≥1,200,000 lux·hr (total illuminance), ≥200 W·h/m² (total near-ultraviolet radiation energy)	2	Unpackaged glass vial or a glass vial placed in a paper boxb)
	40 mg/1 mL				
	80 mg/0.8 mL	100 mg/mL		1	

a) Samples were placed only in the inverted position; b) The control sample was covered with aluminum foil.

No apparent changes in quality attributes were observed throughout the storage period in the long-term testing.

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Based on the above data and by bracketing, a shelf life of 18 months has been proposed for the drug product when stored at 2 to 8°C in a glass vial protected from light. The long-term testing will be continued for up to 36 months.

2.A.(3) Reference material

The reference material is prepared from the drug substance and stored at ≤ ███°C. A shelf life of 24 months is currently specified. ███████████████████████████████████
███
███
██ The reference material is characterized by MALDI-TOF-MS (assay using intact proteins, free sugar chains, samples from which sugar chains have been removed, or reduced samples after removal of sugar chains), peptide mapping, ICP-MS, sulfhydryl group analysis, N-terminal amino acid sequencing, ESI-TOF-MS (assay using samples from which sugar chains have been removed or non-reduced samples after removal of sugar chains), ultracentrifugation, and CD spectra (far- and near-ultraviolet regions).

2.B Outline of the review by PMDA

Based on the submitted data and the following review, PMDA concluded that the quality of the drug substance and the drug product is adequately controlled.

Establishment of shelf life for the drug product

The proposed drug product is available at a concentration of 40 mg/mL in 4 different fills and at 100 mg/mL in a single fill.

At present, of the 3 batches of the 40 mg/1 mL formulation for long-term stability study, 1 batch has been studied only for 6 months. The applicant commented that a shelf-life of 18 months is feasible for the 40 mg/1 mL formulation similarly to other formulations because stability in 40 mg/mL formulations in other fills has not been affected by a difference in fill.

PMDA considers the shelf-life of the proposed drug product as follows:

There is no problem with the shelf-life of 18 months for the 12 mg/0.3 mL, 18 mg/0.45 mL, 28 mg/0.7 mL, and 80 mg/0.8 mL formulations. A shelf-life for the 40 mg/1 mL formulation should normally be established based on data from all 3 batches stored under the long-term storage conditions. However, PMDA concluded that it is acceptable to set a shelf-life of 18 months also for the 40 mg/1 mL formulation for the following reasons:

- Currently available long-term storage data and other stability study data show no apparent changes in quality attributes of any formulations including the 40 mg/1 mL formulation;
- As the largest among the 4 fills of the 40 mg/mL formulation, the 40 mg/1 mL formulation is unlikely to be inferior in stability to the 12 mg/0.3 mL formulation, which is the smallest fill;
- At present, there are no approved drugs indicated for hypophosphatasia in Japan; therefore, it would be clinically meaningful to make all of the 5 formulations needed for dose adjustment promptly available in clinical settings.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A Summary of the submitted data

Primary pharmacodynamic studies conducted for this application included *in vitro* studies and *in vivo* studies using *Akp2*^{-/-} mice,[8] a hypophosphatasia model in mice in which the tissue non-specific alkaline phosphatase (TNSALP) gene has been inactivated. No secondary pharmacodynamic or pharmacodynamic drug interaction studies have been conducted. In safety pharmacology studies, the effects of asfotase alfa on the central nervous system and the respiratory system were investigated. Effects on the cardiovascular system were evaluated in repeated dose toxicity studies. Effects on serum calcium and phosphorus and acute reactions were also investigated.

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[8] Knockout mice with a mixed genetic background of C57BL36 and 129J mice in which the TNSALP gene was inactivated by homologous recombination (Narisawa S, et al. *Developmental dynamics*. 1997;208:432-46)

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) *In vitro* studies

(a) Binding to hydroxyapatite (4.2.1.1.9)

Hydroxyapatite (HA) and asfotase alfa (5 μg) or bovine kidney-derived TNSALP (5 μg) were incubated in RPMI medium containing 0.1% bovine serum albumin (BSA), and the alkaline phosphatase (ALP) activity in the HA-bound fraction and unbound fraction was measured.[9] The HA binding ratio[10] was 96.0% to 97.0% for asfotase alfa and 3.0% for renal TNSALP.[11]

(b) Inhibition of mineralization in MC3T3-E1 cells (4.2.1.1.10)

Mineralization was induced in MC3T3-E1 cells in the presence of ascorbic acid and beta-glycerophosphate. From Day 6 of cultivation, the cells were incubated untreated, in the presence of inorganic pyrophosphate (PPi), or in the presence of PPi and asfotase alfa (132 U/L). Extracellular matrix accumulation and mineralization on Day 14 of cultivation were evaluated by collagen staining and by calcium assay, respectively. The calcium level in the cells cultured in the presence of PPi alone was decreased compared to that in the untreated cells, demonstrating the inhibition of mineralization by PPi. Compared to the cells cultured in the presence of PPi alone, the calcium level in the cells cultured in the presence of PPi and asfotase alfa recovered to a similar level to that in untreated cells, indicating that asfotase alfa suppresses PPi's inhibition of mineralization. Extracellular matrix accumulation remained unchanged regardless of treatment condition.

3.(i).A.(1).2) *In vivo* study

Akp2^{-/-} mice accumulate the physiological substrates of TNSALP, i.e. PPi, pyridoxal 5'-phosphate (PLP), and phosphoethanolamine, either in plasma or in urine. Approximately 8 days after birth, *Akp2^{-/-}* mice exhibit various degrees of bone matrix hypomineralization, including a normal skeletal phenotype in some cases, in comparison with wild-type mice from the same litter.[12] *Akp2^{-/-}* mice show signs of failure to thrive such as reduced body weight gain and bone shortening and die early (10 to 12 days after birth). These premature deaths are mainly attributed to apnea and epileptic seizures because these events occur

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[9] ALP activity was determined by a colorimetric assay method using *p*-nitrophenyl phosphate (pNPP) as substrate through the calibration curve prepared using a reference material of known concentration. One unit (U) of enzyme activity was defined as the amount of enzyme required to degrade pNPP and to produce 1 μmol p-nitrophenol (pNP) in 1 minute.

[10] ALP activity in the HA-bound fraction/(ALP activity in the HA-unbound fraction + ALP activity in the HA-bound fraction) × 100

[11] Similarly, asfotase alfa (5 μg) or bovine kidney-derived TNSALP (5 μg) was incubated in the absence of HA to measure ALP activity. The total ALP activity in asfotase alfa incubated in the presence of HA (2.661 μg; unbound fraction + bound fraction) decreased by approximately 50% compared to that in the absence of HA (5.054 μg). The applicant commented that there is currently no detailed understanding of the factors that cause this decrease. The total ALP activity in the bovine kidney-derived TNSALP incubated in the absence of HA was 6.325 μg and that incubated in the presence of HA was 6.303 μg, showing no decrease in ALP activity.

[12] According to the applicant's explanation, in a colony of *Akp2^{-/-}* mice at Alexion Pharmaceuticals Inc., 10% to 50% of mice from the same litter exhibited a phenotype associated with normal mineralization. Some *Akp2^{-/-}* mice have been reported to exhibit a normal bone phenotype (Yadav MC, et al. *Bone*. 2011;49:250-6; Millan J, et al. *J Bone Miner Res*. 2008;23:777-87; Narisawa S, et al. *Dev Dyn*. 1997;208:432-46).

usually 1 to 2 days before death.[13] Unless otherwise stated, *Akp2*^{-/-} mice were fed a pyridoxine-supplemented diet in *in vivo* studies.[14]

(a) Prophylactic treatment (9-16 days; 4.2.1.1.2, 4.2.1.1.12, 4.2.1.1.14, 4.2.1.1.15)

Asfotase alfa (8.2 mg/kg/day) or vehicle[15] was subcutaneously administered to *Akp2*^{-/-} mice (n = 15-16/group) once daily for 14 to 15 days from birth, and PPi concentration in plasma was measured at 0 to 96 hours after the last dose.[16] Untreated wild-type mice from the same litter (n = 13-14/group) were also evaluated in a similar manner.[17] The results[18] showed that PPi concentration in plasma at 12 hours after the last dose was significantly increased in *Akp2*^{-/-} mice in the vehicle group compared with the wild-type mice and was significantly decreased in *Akp2*^{-/-} mice in the asfotase alfa group compared with those in the vehicle group (4.2.1.1.14).

Asfotase alfa (8.2 mg/kg/day) or vehicle[15] was subcutaneously administered to *Akp2*^{-/-} mice (n = 30-46/group) once daily for 9 days from birth, and PLP concentration in plasma was measured at 24 hours after the last dose. Untreated wild-type mice from the same litter (n = 10) were also evaluated in a similar manner. Mice were not fed a pyridoxine-supplemented diet in this study. PLP concentration in plasma at 24 hours after the last dose was significantly increased in *Akp2*^{-/-} mice in the vehicle group compared with the wild-type mice, and was significantly decreased in *Akp2*^{-/-} mice in the asfotase alfa group compared with those in the vehicle group (4.2.1.1.15).

Asfotase alfa (8.2 mg/kg/day) or vehicle[19] was subcutaneously administered to *Akp2*^{-/-} mice (n = 19-20/group) once daily for 15 days from birth, and mineralization defects in the left hind leg (metatarsal and phalanx) at 24 hours after the last dose were examined using X-ray images. Severity of mineralization defects was graded into 4 categories[20] on a blind basis. Body weight and the lengths of the left tibia and left femur were measured. Untreated wild-type mice from the same litter (n = 18) were

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[13] Narisawa S, et al. *Dev Dyn*. 1997;208:432-46; Millán JL. *Mammalian Alkaline Phosphatases: From Biology to Applications in Medicine and Biotechnology*. 2006;Weinheim, Germany: Wiley-VCH Verlag GmbH & Co, Chapter 8:131-64; Millan JL, et al. *Purinergic Signalling*. 2006;2:335-41; Millan JL, et al. *J Bone Miner Res*. 2008;23:777-87

[14] PLP, an active form of vitamin B$_6$ and an *in vivo* substrate for TNSALP, functions as a coenzyme required for various enzyme reactions in the body. PLP is converted to pyridoxal by ALP and is taken into tissues/cells; hence, a TNSALP deficiency is considered to cause vitamin B$_6$ deficiency in tissues/cells, leading to neurotransmitter biosynthesis inhibition in central nervous system (e.g., decreased glutamate decarboxylase activity in gamma-aminobutyric acid synthesis) and resulting vitamin B$_6$-dependent convulsive seizures. *Akp2*^{-/-} mice were fed pyridoxine-supplemented diet ad lib in *in vivo* studies to prevent premature deaths from convulsive seizures due to defective vitamin B$_6$ metabolism.

[15] 25 mM sodium phosphate containing 150 mM sodium chloride (pH 7.4)

[16] Plasma PPi concentration was measured in *Akp2*^{-/-} mice at 0 and 12 hours after the last dose in the vehicle group and at 12, 24, 48, and 96 hours after the last dose in the asfotase alfa group.

[17] Plasma PPi concentration was measured in wild-type mice at time points that corresponded to 0, 12, and 96 hours after the last dose in *Akp2*^{-/-} mice.

[18] One of 15 *Akp2*^{-/-} mice in the vehicle group and 1 of 16 *Akp2*^{-/-} mice in the asfotase alfa group died at 0 and 96 hours post-dose, respectively.

[19] 25 mM sodium phosphate containing 150 mM sodium chloride, 0.1 mM magnesium chloride, and 20 μM zinc chloride (pH 7.4)

[20] Severe: marked morphological defect with complete absence of the inner and side phalanges with no secondary ossification centers
Moderate: completely formed phalanges with vague secondary ossification centers
Mild: completely formed phalanges with unstable and defective secondary ossification centers
Normal: completely formed phalanges with intact secondary ossification centers

also evaluated in a similar manner. As a result,[21] severe, moderate, and mild mineralization defects, and normal mineralization patterns were seen in 5, 7, 0, and 6 of 18 *Akp2*^{-/-} mice, respectively, in the vehicle group, and 0, 7, 4, and 8, respectively, of 19 *Akp2*^{-/-} mice in the asfotase alfa group, demonstrating that asfotase alfa tended to alleviate the severity of defects. Body weight gain was significantly reduced on and after Day 6 in *Akp2*^{-/-} mice in the vehicle group compared with wild-type mice, while body weight was significantly increased on and after Day 11 in *Akp2*^{-/-} mice in the asfotase alfa group compared with those in the vehicle group. The lengths of the left tibia and left femur (mean ± standard deviation [SD]) were significantly shortened in *Akp2*^{-/-} mice in the vehicle group (11.71 ± 1.06 mm; 8.58 ± 0.77 mm) compared with wild-type mice (13.06 ± 0.59 mm; 9.43 ± 0.39 mm), and were significantly longer in *Akp2*^{-/-} mice in the asfotase alfa group (12.59 ± 0.75 mm; 9.18 ± 0.42 mm) compared with those in the vehicle group (4.2.1.1.2).

Asfotase alfa (8.2 mg/kg/day) or vehicle[15] was subcutaneously administered to *Akp2*^{-/-} mice (n = 29-30/group) once daily for 16 days from birth, and bone mass in the fibrocartilage region of the calcaneus-Achilles tendon and around the secondary ossification center was examined using micro CT on the day after the last dose. Untreated wild-type mice from the same litter (n = 3) were also evaluated in a similar manner. The results[22] showed that bone mass (mean ± SD) was significantly decreased in *Akp2*^{-/-} mice in the vehicle group (0.0039 ± 0.0062 mm^3) compared with wild-type mice (0.080 ± 0.015 mm^3), and was significantly increased in *Akp2*^{-/-} mice in the asfotase alfa group (0.020 ± 0.015 mm^3) compared with those in the vehicle group (4.2.1.1.12).

(b) Prophylactic treatment (43-52 days; 4.2.1.1.1, 4.2.1.1.3, 4.2.1.1.4, 4.2.1.1.7, 4.2.1.1.8, 4.2.1.1.13)

Asfotase alfa or vehicle was subcutaneously administered at various dosage to *Akp2*^{-/-} mice for 43 to 52 days from birth, and mineralization defects in the left hind leg were evaluated at 24 to 48 hours after the last dose or at death. Untreated wild-type mice from the same litter were also evaluated in a similar manner.[23] The severities[20] of mineralization defects and the duration of survival are shown in Table 4.

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[21] One of 19 *Akp2*^{-/-} mice in the vehicle group and 1 of 20 *Akp2*^{-/-} mice in the asfotase alfa group died.

[22] Twenty-one of 30 *Akp2*^{-/-} mice in the vehicle group and 2 of 29 *Akp2*^{-/-} mice in the asfotase alfa group died.

[23] Untreated wild-type mice from the same litter (n = 15 in 4.2.1.1.1, n = 25 in 4.2.1.1.3, n = 32 in 4.2.1.1.4, n = 30 in 4.2.1.1.5, and n = 33 in 4.2.1.1.7) were also evaluated in a similar manner, but the results are omitted here.

Table 4. Severity of mineralization defects and duration of survival after prophylactic treatment with asfotase alfa

	Dose (mg/kg)	Regimen	Dose based on ALP activity[d] (U/kg/day)	Treatment duration (days)	n	Severity of mineralization defects				Survival duration (days)
						Severe	Moderate	Mild	Normal	
4.2.1.1.1	0[a]	Once daily	0	52	16	NS[e]				18.5
	8.2	Once daily	2452	52	17		0/13 (0.0)		13/13 (100.0)	≥52
4.2.1.1.3	8.2	Once every 3 days	682	52	22	2/21 (9.5)	4/21 (19.0)	2/21 (9.5)	13/21 (61.9)	26
	8.2	Once weekly	303	52	19	5/19 (26.3)	7/19 (36.8)	4/19 (21.1)	3/19 (15.8)	24
4.2.1.1.4	4.3	Once daily	3788	43	18	0/18 (0.0)	2/18 (11.1)	1/18 (5.6)	15/18 (83.3)	39
	15.2	Once every 3 days	4493	43	19	0/19 (0.0)	0/19 (0.0)	0/19 (0.0)	19/19 (100.0)	41
	15.2	Once weekly	1938	43	20	0/20 (0.0)	2/20 (10.0)	1/20 (5.0)	17/20 (85.0)	32.5
4.2.1.1.5	0[b]	Once daily	0	43[c]	16	8/16 (50.0)	6/16 (37.5)	0/16 (0.0)	2/16 (12.5)	20
	8.2	Once daily	7211	43	21	0/21 (0.0)	0/21 (0.0)	0/21 (0.0)	21/21 (100.0)	≥43
4.2.1.1.7	0[b]	Once daily	0	43	21	14/20 (70.0)	1/20 (5.0)	3/20 (15.0)	2/20 (10.0)	19
	0.5	Once daily	503	43	18	3/18 (16.7)	4/18 (22.2)	4/18 (22.2)	7/18 (38.9)	24
	2.0	Once daily	2010	43	20	0/20 (0.0)	3/20 (15.0)	0/20 (0.0)	17/20 (85.0)	30.5
	8.2	Once daily	8241	43	19	0/19 (0.0)	0/19 (0.0)	0/19 (0.0)	19/19 (100.0)	≥43

n = number of mice
Severity of mineralization defects, number of mice/number of mice analyzed (%); survival duration, median (days)
4.2.1.1.1, Sixteen of 16 mice in the vehicle group died by Day 24. In this study, based on the following definitions, the severity of mineralization defects was assessed on a blind basis as being either as defective (apparent morphological defect, lack of phalange(s), or formation of phalanges with no secondary ossification centers) or normal (complete formation of phalanges with intact secondary ossification centers).
4.2.1.1.3, In the asfotase alfa groups, 21 of 22 mice treated once every 3 days and 19 of 19 mice treated once weekly died.
4.2.1.1.4, In the asfotase alfa groups, 10 of 18 mice treated once daily, 12 of 19 mice treated once every 3 days, and 17 of 20 mice treated once weekly died.
4.2.1.1.5, One of $Akp2^{-/-}$ 21 mice in the asfotase alfa group and 16 of 16 $Akp2^{-/-}$ mice in the vehicle group died. In the 4.2.1.1.5 study in which asfotase alfa (8.2 mg/kg/day) or vehicle was subcutaneously administered to $Akp2^{-/-}$ mice (n = 16-17/group) once daily for 29 to 31 days from 15 days after birth or asfotase alfa (8.2 mg/kg/day) was subcutaneously administered to $Akp2^{-/-}$ mice (n = 21) once daily for 43 to 45 days from birth, $Akp2^{-/-}$ mice treated with vehicle from 15 days after birth were defined as the vehicle group and those treated with asfotase alfa from birth were defined as the asfotase alfa group [see "(d) Curative treatment"].
4.2.1.1.7, Twenty-one of 21 $Akp2^{-/-}$ mice in the vehicle group and the following numbers of mice in the asfotase alfa groups died: 18 of 18 $Akp2^{-/-}$ mice in the 0.5 mg/kg group, 16 of 20 $Akp2^{-/-}$ mice in the 2.0 mg/kg group, and 1 of 19 $Akp2^{-/-}$ mice in the 8.2 mg/kg group.
a) 25 mM sodium phosphate containing 150 mM sodium chloride, 0.1 mM magnesium chloride, and 20 μM zinc chloride (pH 7.4)
b) 25 mM sodium phosphate containing 150 mM sodium chloride (pH 7.4)
c) Vehicle was administered once daily from 15 days after birth for 29 to 31 days.
d) Daily dose normalized by ALP activity of each batch of asfotase alfa used in the studies
e) No data are available because no X-rays were taken in the vehicle group

The pooled data from primary pharmacodynamic studies were analyzed to investigate the relationship between the dose and efficacy of asfotase alfa.[24] Bone mineralization defects were graded according to the severity criteria based on X-ray images. It was suggested that a sigmoid E_{max} model dependent on the ALP activity (U/kg/day) equivalent dose of asfotase alfa can be fitted to the proportion of mice showing a normal mineralization pattern. The ALP activity equivalent dose producing the E_{max} in 50% of the population (ED_{50}) was 743 U/kg, and the proportion of mice showing a normal mineralization pattern appeared to reach the peak when the dose was 2000/U/kg/day. Furthermore, the 75% survival duration[25] appeared to be consistent with a linear regression model, which showed that the survival duration was prolonged in a manner dependent on the dose (mg/kg/day) of asfotase alfa (4.2.1.1.8).

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[24] The analysis was performed using the results of the studies shown in Table 4 and data from $Akp2^{-/-}$ mice in the vehicle group in the study described in 4.2.1.1.6, in which asfotase alfa (once daily at 8.2 mg/kg, once every 3 days at 24.6 mg/kg, or once weekly at 57.4 mg/kg) or vehicle (once weekly) was subcutaneously administered to $Akp2^{-/-}$ mice (n = 17-20/group) for 35 to 36 days from 12 days after birth [see "(d) Curative treatment"].
[25] The period during which 75% of population remained alive

Asfotase alfa (0.5, 2.0, or 8.2 mg/kg/day) or vehicle[15] was subcutaneously administered to $Akp2^{-/-}$ mice (n = 18-21/group) once daily for 43 days from birth, and body weight was monitored. Untreated wild-type mice from the same litter (n = 33) were also evaluated in a similar manner. $Akp2^{-/-}$ mice in the vehicle group showed a significant reduction in body weight gain compared with wild-type mice on and after Day 7. A significant increase in body weight was observed in $Akp2^{-/-}$ mice in the asfotase alfa 0.5 g/kg group on and after Day 18 and in those in the 2.0 or 8.2 mg/kg group on and after Day 8 compared with the vehicle group. The body weight in $Akp2^{-/-}$ mice in the asfotase alfa 8.2 mg/kg group was similar to that in wild-type mice at any time point (4.2.1.1.7).

The effect of asfotase alfa on the lengths of the left tibia and left femur was not evaluated because all the $Akp2^{-/-}$ mice in the vehicle group had died by the end of the respective studies (4.2.1.1.1, 4.2.1.1.5, and 4.2.1.1.7).

Asfotase alfa (4 mg/kg/day)[26] or vehicle[15] was subcutaneously administered to $Akp2^{-/-}$ mice (n = 20/group) once daily for 43 days from birth, and bone mass in the fibrocartilage region of the calcaneus-Achilles tendon and around the secondary ossification center was examined using micro CT at 24 hours after the last dose. Untreated wild-type mice from the same litter (n = 10) were also evaluated in a similar manner. The results[27] showed that bone mass (mean ± SD) was significantly decreased in $Akp2^{-/-}$ mice in the vehicle group (0.0054 ± 0.017 mm^3) compared with wild-type mice (0.18 ± 0.029 mm^3), and was significantly increased in $Akp2^{-/-}$ mice in asfotase alfa group 1 (0.051 ± 0.026 mm^3) and in asfotase alfa group 2 (0.066 ± 0.029 mm^3) compared with the vehicle group (4.2.1.1.13).

(c) Termination of prophylactic treatment (4.2.1.1.16)

Asfotase alfa (8.2 mg/kg/day) was subcutaneously administered to $Akp2^{-/-}$ mice (n = 28) once daily for 35 days from birth. Body weight and physical condition were monitored from the end of treatment until death. Mineralization defects in the left hind leg were examined at death. Untreated wild-type mice from the same litter (n = 32) were also evaluated in a similar manner. In this study, mice were not fed on a pyridoxine-supplemented diet. All the $Akp2^{-/-}$ mice in the asfotase alfa group died within 53 days after birth (18 days after the last dose), and the median survival after the last dose was 14 days. In the severity assessment[20] of mineralization defects at death, all the $Akp2^{-/-}$ mice in the asfotase alfa group and wild-type mice were assessed as normal with no aggravated cases after termination of administration. Decreased body weight and aggravated clinical signs (decreased activity, dehydration, forward rounded posture, and epileptic seizures) were observed 7 days after the last dose and thereafter in $Akp2^{-/-}$ mice in the asfotase alfa group.

(d) Curative treatment (4.2.1.1.5, 4.2.1.1.6)

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[26] In the study, $Akp2^{-/-}$ mice were divided into 2 asfotase alfa groups (asfotase alfa groups 1 and 2) in which 2 different batches were used.

[27] Twenty of 20 $Akp2^{-/-}$ mice in the vehicle group, 8 of 20 $Akp2^{-/-}$ mice in the asfotase alfa group 1, and 2 of 20 $Akp2^{-/-}$ mice in the asfotase alfa group 2 died.

Asfotase alfa (8.2 mg/kg/day) or vehicle[15] was subcutaneously administered to $Akp2^{-/-}$ mice (n = 16-17/group) once daily for 29 to 31 days from 15 days after birth, and mineralization defects in the left hind leg were examined on the day after the last dose or at death. Body weight was also monitored. Untreated wild-type mice from the same litter (n = 30) were evaluated in a similar manner. The results[28] for mineralization defects showed that those assessed as severe, moderate, mild, and normal were seen in 8, 6, 0, and 2, respectively, of 16 $Akp2^{-/-}$ mice in the vehicle group and 9, 0, 1, and 7, respectively, of 17 $Akp2^{-/-}$ mice in the asfotase alfa group, while all the wild-type mice were assessed as normal. Body weight gain was significantly reduced in $Akp2^{-/-}$ mice in the vehicle group compared to wild-type mice throughout the administration period after the first dose (15 days after birth). In $Akp2^{-/-}$ mice, body weight continued to decrease until death in the vehicle group, and continued to increase in the asfotase alfa group from 4 to 5 days after the first dose (19-20 days after birth) until the end of the study. However, the body weight in the asfotase alfa group was lower than that in wild-type mice by approximately 20% at the end of the study (44 days after birth). The median survival in the asfotase alfa group was 39 days, which was significantly longer than that in the vehicle group (20 days). A similar evaluation was performed in $Akp2^{-/-}$ mice (n = 21) that received repeated subcutaneous doses of asfotase alfa at 8.2 mg/kg/day once daily from birth for 43 to 45 days [see Table 4 in "(b) Prophylactic treatment (43-52 days)"].

Asfotase alfa (once daily at 8.2 mg/kg/day, once every 3 days at 24.6 mg/kg, or once weekly at 57.4 mg/kg) or vehicle[15] (once weekly) was subcutaneously administered to $Akp2^{-/-}$ mice (n = 17-20/group) for 35 days from 12 days after birth,[29] and mineralization defects in the left hind leg were examined at 24 hours after the last dose or at death. Body weight was also monitored. Untreated wild-type mice from the same litter (n = 39) were evaluated in a similar manner. Mineralization defects[30] (severe, moderate, mild, and normal) were found in 10, 1, 0, and 6 of 17 $Akp2^{-/-}$ mice, respectively, in the vehicle group; in 6, 1, 0, and 12 of 19 $Akp2^{-/-}$ mice, respectively, in the group of animals receiving asfotase alfa once daily; in 9, 1, 0, and 10 of 20 $Akp2^{-/-}$ mice, respectively, in the group of animals receiving asfotase alfa once every 3 days; and in 10, 1, 0, and 7 of 18 $Akp2^{-/-}$ mice, respectively, in the group of animals receiving asfotase alfa once weekly. Body weight gain in $Akp2^{-/-}$ mice in the vehicle group was significantly reduced compared to that in wild-type mice throughout the administration period after the first dose (12 days after birth). Body weight in $Akp2^{-/-}$ mice in all asfotase alfa groups continued to increase until the end of the study. The median survival in any asfotase alfa groups (38 days in the once daily regimen group, 30.5 days in the once every 3 day regimen group, and 21 days in the once weekly regimen group) was significantly longer than that in the vehicle group (20 days).

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[28] Death occurred in 6 of 39 $Akp2^{-/-}$ mice before starting administration of asfotase alfa or vehicle (1-15 days after birth), and in 16 of 16 in the vehicle group and 14 of 17 $Akp2^{-/-}$ mice in the asfotase alfa group during the period from the start of administration to the end of the study. When all the $Akp2^{-/-}$ mice died, wild-type mice from the same litter (14 of 30) were sacrificed moribund.

[29] $Akp2^{-/-}$ mice received a subcutaneous dose of asfotase alfa at 8.2 mg/kg/day once daily for the first 7 days of administration and then according to the assigned respective dosage regimens on Day 8 onward.

[30] The number of $Akp2^{-/-}$ mice that died by the end of the study was as follows: 17 of 17 mice in the vehicle group; 18 of 18 mice in the asfotase alfa once daily regimen group, 17 of 20 mice in the once every 3 days regimen group, and 15 of 19 mice in the once weekly regimen group. When all the $Akp2^{-/-}$ mice died, wild-type mice from the same litter (32 of 39) were withdrawn from the study.

3.(i).A.(2) Safety pharmacology

3.(i).A.(2).1) Central nervous system (4.2.1.3.2)

A single dose of asfotase alfa (3, 30, or 88 mg/kg) or vehicle[15] was intravenously administered to male rats (n = 10/group[31]), and the effect of treatment on the central nervous system was evaluated by the functional observation battery (FOB) before dosing and at 0, 0.5, 6, and 24 hours after dosing. In the asfotase alfa 30 and 88 mg/kg groups, decreased activity, decreased response to stimuli, abnormal gait, decreased extensor thrust reflex, altered landing foot splay, weakness of limbs, decreased body temperature, and irregular/labored breathing were observed at 0 to 0.5 hour after dosing, but resolved within 6 hours after dosing. Swelling and reddish or bluish discoloration of the limb were observed at the time of resolution of the above abnormalities, but resolved within 24 hours after dosing. In the asfotase alfa 3 mg/kg group, reddish discoloration of the limb occurred immediately after dosing in 2 of 10 rats but with no behavioral effects.

3.(i).A.(2).2) Respiratory system (4.2.1.3.3)

A single dose of asfotase alfa (3, 30, or 90 mg/kg) or vehicle[15] was intravenously administered to male rats (n = 8/group), and the effect of treatment was evaluated by measuring tidal volume, minute ventilation, and respiratory rate before dosing and at 2, 6, and 24 hours after dosing using plethysmography. As a result, respiratory rates in the asfotase alfa 30 and 90 mg/kg groups were significantly decreased mainly at 2 hours after dosing, compared with the vehicle group. A decrease from baseline in respiratory rate resulted from decreased minute ventilation was observed in all the asfotase alfa groups, being most serious in the 90 mg/kg group. This hypoventilation effect was sporadically observed up to 24 hours after dosing.

The applicant explained that these acute reactions in rats after intravenous administration of asfotase alfa were of little toxicological significance [see "4.(iii).B.(3).2) Injection-related reactions"].

3.(i).A.(2).3) Cardiovascular system

Twenty six-week repeated subcutaneous dose toxicity study in monkeys (4.2.3.2.5)

Repeated doses of asfotase alfa (0.43, 2.14, or 10 mg/kg/day) or vehicle[15] were subcutaneously administered to male and female unanesthetized monkeys (n = 5/sex/group) once daily for 26 weeks, and limb-lead electrocardiographic (ECG) data were collected at baseline, and before dosing and at 0.25, 4, 12, and 24 hours after dosing at Weeks 1 and 26. No changes were detected in heart rate or ECG parameters (PR, RR, QRS, QT, and QTc[32] intervals).

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[31] Toxicokinetics of the respective doses was also evaluated using different rats.
[32] Fridericia's correction formula

The serum asfotase alfa concentrations ($C_{max,ss}$ and $AUC_{0-168\,h.ss}$) after administration at 10 mg/kg to monkeys were 6710 U/L and 872,000 U·h/L, respectively,[33] which was approximately 2.1-fold and 2.5-fold higher than those after administration at the clinical dose.[34]

3.(i).A.(2).4) Effects on serum phosphorus and calcium (4.2.1.3.1: reference data)

Asfotase alfa is effective in promoting mineralization, and therefore may induce convulsive seizures resulting from hypocalcemia.[35] This possibility was investigated in *Akp2*^{-/-} mice (n = 19-20/group) that received asfotase alfa subcutaneously as a single dose (8.2 mg/kg) or repeated doses (8.2 mg/kg/day for 4 days) from 12 days after birth. Serum calcium and phosphorus levels were measured at 4 hours after a single dose or the last dose of the repeated administration. Also, untreated *Akp2*^{-/-} mice (n = 19; control group) and wild-type mice from the same litter (n = 17) were evaluated in a similar manner. The results[36] showed no marked differences in serum calcium and phosphorus levels at baseline (12 days after birth) between *Akp2*^{-/-} mice in the control group (11.33 ± 1.77 mg/dL and 10.61 ± 1.46 mg/dL, respectively) and wild-type mice (10.64 ± 0.82 mg/dL and 11.18 ± 1.15 mg/dL, respectively). The serum calcium and phosphorus levels 4 hours post-dose in *Akp2*^{-/-} mice were 11.32 ± 1.00 mg/dL and 12.42 ± 3.40 mg/dL, respectively, in the single-dose group, and those 4 hours after the last dose of repeated administration were 11.33 ± 1.53 mg/dL and 12.13 ± 3.79 mg/dL, respectively, in the repeat-dose group. Neither group showed any marked decrease in serum calcium or phosphorus levels compared with those in the control group.[37]

3.(i).A.(2).5) Acute reactions (4.2.1.3.4: reference data)

Acute reactions (e.g., swelling and discoloration of the limbs, decreased activity) were observed after intravenous administration of asfotase alfa to rats. Therefore, the involvement of inflammation, histamine, complements, and tryptase in such acute reactions was investigated in male rats (n = 5-6/group) that received a single dose of asfotase alfa (90 or 180 mg/kg) or vehicle[15] under various conditions including injection rate (bolus, over 15 minutes, or over 30 minutes), route of administration (intravenous or subcutaneous), and pretreatment (subcutaneous or intraperitoneal administration of diphenhydramine [DP] or subcutaneous administration of dexamethasone [Dex]).[38] Clinical signs

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[33] Exposure on the last day after the last dose of 10 mg/kg of asfotase alfa in the 26-week repeated subcutaneous administration toxicity study in monkeys (4.2.3.2.5)

[34] The estimated steady-state exposure in a typical patient 1 week after administration at Week 72, where a typical patient was defined as a patient with a body weight of 50 kg in whom PK was not affected by immunogenicity after administration of asfotase alfa (batch size, ▮ L) containing ▮ mol of sialic acid per mol of protein and having ▮ U/mg of enzyme activity. The $AUC_{0-168\,h}$ was calculated using the following formula: (bioavailability of ▮ L batch) × (total weekly dose)/(mean total body clearance). $C_{max,ss}$ indicates the maximum steady-state activity during the 7 days after administration.

[35] Latus J, et al. *Int J Nephrol Renovasc Dis*. 2013;6:131-7; Witteveen JE, et al. *Eur J Endocrinol*. 2013;168:R45-53

[36] Three of 20 *Akp2*^{-/-} mice in the repeat-dose group died 12 to 14 days after birth. The applicant explained that these deaths had been expected from the pathological condition of *Akp2*^{-/-} mice.

[37] A significant increase in serum phosphorus level (17%) was observed in *Akp2*^{-/-} mice in the single-dose asfotase alfa group compared with the vehicle group. The applicant explained that this result had been expected from hydrolysis of PPi, a pharmacological action of asfotase alfa.

[38] In Experiment 1, rats underwent one of the following 9 treatments: (1) intravenous bolus administration of vehicle (without pretreatment), (2) intravenous bolus administration of vehicle (subcutaneous pretreatment with DP at 10 mg/kg), (3) intravenous bolus administration of asfotase alfa at 90 mg/kg (without pretreatment), (4) intravenous bolus administration of asfotase alfa at 90 mg/kg (subcutaneous pretreatment with DP at 10 mg/kg), (5) continuous intravenous administration of vehicle over 15 minutes (without pretreatment), (6)

(piloerection, swelling of the face or limbs, decreased activity, excessive scratching behavior, and skin discoloration) in the rats were evaluated at 0, 0.25, 1, 2, 4, 6, and 24 hours after dosing.[39] In addition, levels of C3, C5, and total complement activity in serum and C3, C4, C5a, and tryptase in plasma were measured.[40] Acute reactions (e.g., piloerection, swelling of the face or limbs, decreased activity) were observed in all the rats receiving an intravenous dose of asfotase alfa, but resolved within 24 hours after dosing. When examined at 2 to 6 hours post-dose, clinical signs observed after continuous intravenous administration of asfotase alfa over 15 or 30 minutes tended to be milder compared with those after intravenous bolus administration. Subcutaneous pretreatment with DP or Dex tended to be effective in reducing the severity of clinical signs at 6 hours after intravenous bolus administration of asfotase alfa. Meanwhile, subcutaneous pretreatment with DP did not reduce the severity of clinical signs after continuous intravenous administration of asfotase alfa over 15 minutes. Intravenous bolus administration or continuous intravenous administration of asfotase alfa over 15 minutes had no effect on serum complement activity level or plasma complement level other than plasma tryptase level, which was increased compared with the level after intravenous bolus administration of vehicle.

3.(i).B   *Outline of the review by PMDA*

The mechanism of action of asfotase alfa

PMDA asked the applicant to explain the mechanism of action of asfotase alfa on the basis of its structure and the differences from human TNSALP.

The applicant responded as follows:

Hypophosphatasia is a hereditary disorder caused by loss-of-function mutations in the gene encoding TNSALP.[1] There are 4 human ALP isoforms of intestinal, placental, germ-cell-specific and tissue non-specific isoenzymes, all of which catalyze the hydrolysis of phosphate monoesters, including PPi, PLP, and phosphoethanolamine, as endogenous substrates. PPi inhibits bone mineralization, and inorganic phosphate (Pi) generated by the hydrolysis of PPi binds to calcium to form HA crystals. ALP thus plays an important role in regulation of bone mineralization. ALP also converts PLP into pyridoxal, which is taken up by cells and tissues, and serves, in the form of active vitamin B_6, as a coenzyme for various endogenous enzymatic reactions. TNSALP is widely present in the body, and is highly expressed in the

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continuous intravenous administration of asfotase alfa at 90 mg/kg over 15 minutes (without pretreatment), (7) continuous intravenous administration of asfotase alfa at 90 mg/kg over 30 minutes (without pretreatment), (8) subcutaneous administration of vehicle (without pretreatment), and (9) subcutaneous administration of asfotase alfa at 180 mg/kg (without pretreatment).

In Experiment 2, rats underwent one of the following 8 treatments: (1) intravenous bolus administration of vehicle (subcutaneous pretreatment with DP at 20 mg/kg), (2) intravenous bolus administration of asfotase alfa at 90 mg/kg (without pretreatment), (3) intravenous bolus administration of asfotase alfa at 90 mg/kg (subcutaneous pretreatment with DP at 20 mg/kg), (4) intravenous bolus administration of asfotase alfa at 90 mg/kg (intraperitoneal pretreatment with DP at 20 mg/kg), (5) continuous intravenous administration of vehicle over 15 minutes (subcutaneous pretreatment with DP at 20 mg/kg), (6) continuous intravenous administration of asfotase alfa at 90 mg/kg over 15 minutes (without pretreatment), (7) continuous intravenous administration of asfotase alfa at 90 mg/kg over 15 minutes (subcutaneous pretreatment with DP at 20 mg/kg), and (8) intravenous bolus administration of asfotase alfa at 90 mg/kg (subcutaneous pretreatment with Dex at 5 mg/kg).

[39] Clinical signs were scored based on the point scales of incidence (0, not occurred; 1, occurred) and severity (1, mild; 2, moderate; 3, severe), and then the sum of the scores were evaluated.

[40] Plasma complement and tryptase concentrations were measured at 0.25 and 24 hours post-dose in 3 of 6 rats in each group of Experiment 1.

liver, kidneys, and bone.[41] The bone TNSALP is expressed in the cell membrane of osteoblasts and chondrocytes and in the matrix vesicle membrane, and mediates PPi degradation in bone mineralization. The liver TNSALP is expressed in biliary canalicular cells and endothelial cells of the liver, and has been reported to mediate endotoxin dephosphorylation, but no liver disease due to the lack of the liver TNSALP has been reported. TNSALP is usually anchored to the cell membrane by a glycosylphosphatidylinositol linkage, but it has been reported that part of the bone and liver TNSALP is released into the blood and circulates throughout the entire body, accounting for about 95% of blood ALP activity.[42] The kidney TNSALP is expressed in the proximal renal tubule, and although its function is as yet not fully elucidated, the presence of nephrocalcinosis in some patients with hypophosphatasia suggests the possible involvement of the kidney TNSALP in mineralization mediated by regional PPi metabolism.[43] In hypophosphatasia, elevated PPi levels suppress HA formation and inhibit bone mineralization, thereby causing rickets or osteomalacia, sometimes accompanied by hypercalcemia and hyperphosphatemia. Also the impairment of PLP regulation results in vitamin B_6 deficiency in cells and tissues, which is implicated in vitamin B_6-dependent convulsive seizures due to the inhibition of neurotransmitter biosynthesis in the central nervous system (for example, a decrease in the activity of glutamate decarboxylase required for gamma-aminobutyric acid synthesis).

Asfotase alfa is a homodimer of a soluble glycoprotein of 726 amino acids, consisting of the catalytic domain of human TNSALP, the Fc domain of human immunoglobulin (Ig) G1, and a deca-aspartate peptide. The soluble catalytic domain of human TNSALP shares the same amino acid sequence and *N*-linked glycosylation sites with endogenous TNSALP. Intravenous ALP injection improved symptoms of hypophosphatasia, but not radiographic findings of the bone. These findings suggest the importance of localization of TNSALP in the bone for effective bone mineralization.[44] The deca-aspartate peptide binds to HA, the inorganic component of the bone, and is therefore expected to improve tissue-selective delivery to the bone (4.2.1.1.9).[45] The enzymatic activity of asfotase alfa to release Pi from the substrate PPi was measured to be 106.2/s with a Michaelis constant of 38.4 μmol/L (3.2.S.1.3). It has also been suggested that asfotase alfa lessens PPi-induced inhibition of mineralization in osteoblast-like MC3T3-E1 cells (4.2.1.1.10). In *in vivo* studies, prophylactic and curative treatment with asfotase alfa in *Akp2*^{-/-} mice, an animal model of hypophosphatasia, improved bone mineralization, growth, and survival. Human and mouse TNSALP have been shown to share 92% amino acid sequence homology, preserving catalytic domain, domain exchange elements required for activity, and secondary structural elements required for dimerization. Therefore, the activity of asfotase alfa is considered to be conserved among animal species.[46] In addition, the tissue distribution and function of TNSALP are believed to be similar between humans and mice.[41] There are no differences between animal species regarding the molecular structures of the endogenous TNSALP substrates, and TNSALP is enzymatically active as a membrane-

[41] Buchet R, et al. *Methods in Molecular Biology*. 2013;1053:27-51

[42] Magnusson P, et al. *J Bone Miner Res*. 1997;12:210-20

[43] Belachew D, et al. *JIMD Rep*. 2013;11:11-24; Baumgartner Sigl S, et al. *Bone*. 2007;40:1655-61

[44] Whyte PM, et al. *J Pediatr*. 1982;101:379-86; Whyte PM, et al. *J Pediatr*. 1984;105:926-33

[45] Kasugai S, et al. *J Bone Miner Res*. 2000;15:936-43

[46] Kozlenkov A, et al. *J Biol Chem*. 2002;277:22992-9; Millan JL, et al. *Purinergic Signal*. 2006;2:335-41; Wollscheid B, et al. *Nat Biotechnology*. 2009;27:378-86; Weiss MJ, et al. *J Biol Chem*. 1988;263:12002-10; Le Du MH, et al. *J Biol Chem*. 2001;276:9158-65

anchored protein. Therefore the activity of TNSALP is thought to be independent of factors such as receptor activity.

On the basis of the above discussions, asfotase alfa is expected to normalize the TNSALP activity mainly in the bone and to reduce accumulated levels of endogenous substrates, and thus, to correct bone mineralization defects.

PMDA accepted the applicant's response.

3.(ii)   Summary of pharmacokinetic studies

3.(ii).A   Summary of the submitted data

The pharmacokinetics of asfotase alfa was evaluated in mice, rats, rabbits, and monkeys that received asfotase alfa or ^{125}I-asfotase alfa as a single intravenous or subcutaneous dose or repeated subcutaneous doses. The pharmacokinetics of repeated doses of asfotase alfa was also evaluated on the basis of toxicokinetics in the repeated dose toxicity studies in rats, rabbits, and monkeys. Serum concentrations of asfotase alfa were quantified by an enzyme activity assay using *p*-nitrophenyl phosphate as substrate, and the lower limit of quantification was 75 or 100 ng/mL in rats, 100 ng/mL in rabbits, and 75 ng/mL in monkeys. Anti-asfotase alfa antibodies in serum were detected by a bridging ELISA, and radioactivity levels in biological samples were determined by a radioactive tracer technique. The results of the principal studies are described below.

3.(ii).A.(1) Absorption (4.2.2.2.1, 4.2.2.2.3-4.2.2.2.6, 4.2.3.2.3-4.2.3.2.5, 4.2.3.5.2.4, 4.2.3.5.3.1, 4.2.3.6.1)

Table 5 shows the serum concentrations of asfotase alfa and pharmacokinetic parameters in mice, rats, rabbits, and monkeys after a single intravenous or subcutaneous dose of asfotase alfa.

Table 5. Pharmacokinetic parameters after a single intravenous or subcutaneous dose of asfotase alfa

Animal species	Sex	Route of administration	Dose (mg/kg)	n/time point	C_{max} (mg/L)	$AUC_{0-\infty}$ (mg·h/mL)	AUC_{last}[a] (mg·h/L)	T_{max} (h)	$T_{1/2}$ (h)	CL (L/h/kg)	Vz[b] (L/kg)
Mice[c]	M	I.V.	2	3-6	26.4	286	258	–	15.61	0.007	–
	M	S.C.	2	3-6	3.07	161	100	12.00	31.08	0.012	–
Akp2^{-/-} mice[c]	F M	S.C.	8.2	4	7.51	227	213	4	14.0	0.036	1.34
WT mice[c]	F M	S.C.	8.2	4	5.03	155	154	8	25.5	0.053	1.07
Rats	M	I.V.	3	3	22.2 (19.5)	106 (33.2)	93.1 (29.3)	0.25 (0.25, 0.25)	27.3 (23.9)	0.031 (40.0)	1.16 (17.2)
	F	I.V.	3	3	22.1 (17.7)	62.1 (16.3)	53.7 (15.1)	0.25 (0.25, 0.25)	33.8 (48.9)	0.049 (18.0)	1.39 (40.5)
	M	S.C.	3	3	0.42 (47.6)	123 (74.4)	24.5 (46.1)	24 (24, 48)	189 (72.7)	0.038 (79.1)	7.53 (45.3)
	F	S.C.	3	3	0.34 (9.70)	33.1 (46.2)	17.3 (23.1)	24 (12, 24)	51.0 (44.2)	0.11 (63.0)	6.76 (5.8)
Rabbits	F	I.V.	5	3	51.4 (1.80)	89.74, 95.67[d]	102 (23.1)	0.37 (0.35, 0.38)	30.0, 29.4[d]	0.056, 0.052[d]	0.71, 0.59[d]
			50	3	1137 (6.70)	2918 (4.6)	2896 (4.3)	0.42 (0.37, 0.67)	33.6 (15.9)	0.017 (4.5)	0.069 (13.1)
	F	S.C.	5	3	0.93 (2.9)	31.2 (15)	25.9 (16.5)	6.03 (6.00, 6.05)	35.9 (16.9)	0.16 (14.0)	6.78 (3.2)
			50	3	11.0 (43.8)	304 (30.7)	280 (32.6)	12.0 (6.02, 12.02)	29.4 (5.1)	0.18 (28.9)	6.37 (43.4)
Monkeys	F	I.V.	3	2	67.2, 70.4[d]	177, 139[d]	152, 130[d]	0.25, 0.25[d]	36.6, 26.2[d]	0.017, 0.022[d]	0.90. 0.82[d]
	F	S.C.	3	2	1.15, 1.06[d]	73.8, 61.3[d]	19.9, 48.0[d]	12.0, 8.0[d]	39.6, 30.4[d]	0.041, 0.049[d]	2.32, 2.15[d]

Mean; Mean (coefficient of variance [%]); T_{max}, median (min, max); –, not calculated;
C_{max}, maximum serum concentration;
$AUC_{0-\infty}$, area under the serum concentration-time curve from time zero to infinity (AUC extrapolated to infinity);
AUC_{last}, area under the serum concentration-time curve from time zero to the time of the last measurable concentration;
T_{max}, time to maximum serum concentration; $T_{1/2}$, elimination half-life; CL, clearance; Vz, volume of distribution;
I.V., intravenous administration; S.C., subcutaneous administration; n, number of animals
a) Mice, $AUC_{0-49 h}$; Akp2^{-/-}/WT mice and rabbits, $AUC_{0-96 h}$; rats and monkeys, $AUC_{0-72 h}$
b) After subcutaneous administration, Vz/F or Vss/F
c) Data from respective time points were pooled for analysis and only mean values of pharmacokinetic parameters were calculated from mean concentration-time profiles.
d) n = 2 (individual values are shown.)

Table 6 shows the pharmacokinetic parameters in rats, monkeys, pregnant rats, and pregnant rabbits after the first dose and the last dose in repeated intravenous or subcutaneous administration of asfotase alfa.

Table 6. Pharmacokinetic parameters after the first dose and the last dose in repeated administration of asfotase alfa

Animal species	Route of administration	Dose (mg/kg)	n	Administration period	C_{max} (mg/mL)		AUC_{last} (mg·h/L)[a]		$T_{1/2}$ (h)[b]	
					First dose	Last dose	First dose	Last dose	First dose	Last dose
Male and female rats	I.V.	3/W	6	4 weeks	16.2 ± 0.652	16.2 ± 1.04	100 ± 2.21	73.1 ± 5.14	18.3	19.8
		30/W	6		298 ± 9.36	147 ± 13.8	869 ± 11.5	567 ± 41.1	15.8	18.5
		90/W	6		567 ± 96.5	678 ± 53.7	1927 ± 75.7	1957 ± 87.0	15.3	23.2
	S.C.[c]	0.84/D	3	4 weeks	0.33 ± 0.087	0.18 ± 0.17	16.6 ± 1.63	6.00 ± 5.77	21.0	–
		8.4/D	3		2.94 ± 0.42	0.22 ± 0.17	137 ± 5.48	6.85 ± 4.28	17.8	12.2
		25.2/D	3		8.00 ± 1.47	0.53 ± 0.24	330 ± 33.0	13.8 ± 5.93	21.4	79.2
	I.V.	1/D	6	26 weeks	6.07 (33.7)	8.37 (62.9)	26.0 (3.4)	16.1 (31.3)	18.3 (57.3)	7.99 (36.1)
		3/D	6		19.3 (27.8)	42.3 (46.2)	58.4 (33.5)	61.9 (38.7)	32.1 (47.5)	8.85 (39.3)
		13/D	6		115 (11.2)	281 (35.2)	364 (3.7)	379 (26.9)	16.0 (2.5)	11.6 (24.4)
Male and female monkeys	I.V.	5/W	10	4 weeks	68.1 (16.1)	53.7 (17.5)	142 (31.2)	105 (33.2)	28.2 (22.6)[d]	39.4 (53.6)[d]
		15/W	10		245 (16.1)	236 (27.1)	606 (12.7)	524 (24.6)	26.4 (20.6)	34.9 (60.4)[e]
		45/W	10		726 (21.5)	691 (12.2)	3190 (20.4)	2670 (20.6)	28.4 (23.8)	23.0 (89.4)[f]
	S.C.	0.43/D	10	26 weeks	0.072 (49.0)	0.36 (34.2)	1.38 (44.0)	21.5 (40.5)	–	61.2 (65.9)[g]
		2.14/D	10		0.45 (20.4)	0.80 (44.6)	8.50 (20.8)	35.1 (61.4)	–	63.0 (48.7)[f]
		10/D	10		2.66 (30.6)	6.68 (34.3)	50.0 (28.6)	303 (27.3)	–	45.3 (37.6)
Pregnant rats	I.V.	10/D	3	14 days	65.9 ± 3.85	36.9 ± 4.87	243 ± 8.95	86.3 ± 10.6	17.8	1.97
		25/D	3		247 ± 8.25	134 ± 14.7	703 ± 34.8	340 ± 53.2	18.1	–
		50/D	3		612 ± 140	672 ± 143	1502 ± 142	1339 ± 117	–	2.11
Pregnant rabbits	I.V.	10/D	3	13 days	121 (34.6)	125 (13.9)	447 (13.5)	321 (43.5)	16.8 (41.2)	3.64 (78.4)
		25/D	3		584 (84.2)	504 (22.4)	1860 (55.4)	1566 (21.5)	10.9 (30.7)	4.07 (70.7)
		50/D	3		1135 (13.2)	1021 (3.2)	4189 (17.6)	3435 (31.4)	8.92, 11.9[h]	2.38, 1.91[h]

Mean ± standard error; Mean (coefficient of variance [%]); –, not calculated;
C_{max}, maximum serum concentration;
AUC_{last}, area under the serum concentration-time curve from time zero to the time of the last measurable concentration;
$T_{1/2}$, elimination half-life; I.V., intravenous administration; S.C., subcutaneous administration; n, number of animals;
W, week; D, day
a) Rats (I.V. [4 weeks]) and monkeys (I.V.), $AUC_{0-72 h}$; rats (S.C.) and monkeys (S.C.), $AUC_{0-96 h}$; rats (I.V. [26 weeks]), pregnant rats, and pregnant rabbits, $AUC_{0-24 h}$
b) Data from respective time points were pooled for analysis and only mean values of $T_{1/2}$ were calculated from mean concentration-time profiles (rats and pregnant rats).
c) Male rats only
d) n = 9; e) n = 7; f) n = 6; g) n = 5; h) n = 2 (individual values are shown)

3.(ii).A.(2) Distribution(4.2.2.2.2, 4.2.2.3.1, 4.2.2.3.2)

Following a single intravenous dose of ^{125}I-asfotase alfa at 5 mg/kg to male juvenile mice (n = 3/time point; radioactivity in blood was measured until 96 hours post-dose), radioactivity peaked at 0.5 hour post-dose in the liver, at 1 hour post-dose in the bone marrow, brain, gallbladder, kidneys, and lungs, at 2 hours post-dose in the calvaria and muscle, and at 8 hours post-dose in the femur and tibia. Radioactivity was detected in all tissues up to 96 hours post-dose, and the mean retention time (MRT_{last}) in the calvaria, femurs, and tibia was ≥40 hours. The ratio of tissue to plasma radioactivity[47] calculated based on AUC was ≥1 in the femurs (6.75), tibiae (1.19), and liver (1.17) and <1 in the kidneys (0.91), gallbladder (0.83), calvaria (0.73), lungs (0.47), muscle (0.13), brain (0.06), and bone marrow (0.02).

Following repeated subcutaneous doses of ^{125}I-asfotase alfa at 4.3 mg/kg to male and female mice (n = 3/time point; radioactivity in blood was measured until 168 hours post-dose), radioactivity peaked at 24 hours post-dose in all organs. Of them, the highest value measured was in the calvaria, followed by the tibiae, liver, femurs, kidneys, lungs, gallbladder, muscle, and brain in descending order. The MRT_{last} in the calvaria, tibiae, femurs, and gallbladder was ≥60 hours. The ratio of tissue to plasma radioactivity[48]

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[47] $AUC_{0-\infty}$ (tissue)/$AUC_{0-\infty}$ (blood) (The ratio in the tibiae was calculated as follows: AUC_{last} [tissue]/$AUC_{0-\infty}$ [blood]). The extrapolated area under the concentration-time curve ($AUC_{0-\infty}$) was calculated to exceed 25%.
[48] AUC_τ (tissue)/AUC_τ (blood)

was ≥1 in the calvaria (1.65) and <1 in the tibiae (0.95), liver (0.79), femurs (0.78), kidneys (0.67), lungs (0.64), gallbladder (0.57), muscle (0.32), and brain (0.11).

Following repeated subcutaneous doses of asfotase alfa once daily at 0.5, 2, or 8.2 mg/kg for 5 days to pregnant mice (gestation days 13-17; n = 8), the serum asfotase alfa concentration (mean ± standard error) at 21 hours post-dose on Day 5 was 0.080 (n = 1), 0.36 ± 0.017, and 1.70 ± 0.059 mg/L in dams and 0.058 ± 0.017, 0.31 ± 0.045, and 1.21 ± 0.26 mg/L in fetuses.

3.(ii).A.(3) Metabolism
No study on metabolism has been conducted.

3.(ii).A.(4) Excretion
No study on excretion has been conducted.

3.(ii).B Outline of the review by PMDA
Accumulation of asfotase alfa

The $AUC_{0-24\,h}$ at Weeks 1, 4, and 17 was increased by approximately 4- to 31-fold in rats that received repeated intravenous doses of asfotase alfa once daily at 1, 3, or 13 mg/kg (4.2.3.2.3) and the $AUC_{0-24\,h}$ at Week 26 was increased by approximately 0.8- to 7-fold compared to that at Week 1 in monkeys that received repeated subcutaneous doses of asfotase alfa once daily at 0.43, 2.14 or 10 mg/kg (4.2.3.2.5). PMDA asked the applicant to explain the accumulation and safety of asfotase alfa in humans, taking account of the accumulation observed in the above once daily administration studies.

The applicant responded as follows:
In the foreign clinical pharmacology study in foreign patients with hypophosphatasia (Study ENB-001-08) who received asfotase alfa subcutaneously at 2 mg/kg as a single dose or repeated doses once weekly for 3 weeks, the maximum plasma concentration (C_{max}) after the first dose and the last dose was 1081 ± 65.2 U/L and 1020 ± 326 U/L, respectively, and the area under the serum concentration-time curve during the dosing interval (AUC_τ) was 138,595 ± 6958 h·U/L and 136,109 ± 41,875 h·U/L, respectively, showing no accumulation of asfotase alfa. In a population pharmacokinetic analysis performed using serum asfotase alfa concentration data of 1183 samples from clinical studies in patients with hypophosphatasia (Studies ENB-001-08, ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10), the accumulation (mean [minimum, maximum]) of asfotase alfa was calculated based on the area under the serum concentration-time curve (AUC) after once weekly administration and was estimated to be 1.14 (1.01, 1.35).

In a pooled analysis of data from clinical studies in patients with hypophosphatasia (Studies ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10), the median total exposure period per subject was 2.5 years, and 48.6% (1314 of 2706 events) of observed adverse events occurred during the

first 24 weeks of treatment, showing no particular increase in adverse events with the increase in administration period.

PMDA accepted the applicant's response.

3.(iii) Summary of toxicology studies
3.(iii).A Summary of the submitted data
Toxicity studies conducted on asfotase alfa include single-dose toxicity, repeat-dose toxicity, reproductive and developmental toxicity, and local tolerance studies. Data from some studies conducted as non-Good Laboratory Practice (GLP) studies were submitted as reference data.

3.(iii).A.(1) Single-dose toxicity
Bolus or drip intravenous infusion toxicity study in monkeys (4.2.3.1.1: reference data)
Male and female juvenile cynomolgus monkeys received asfotase alfa as follows: (i) at 5 or 15 mg/kg via bolus intravenous injection on Days 1 and 8; (ii) at 45, 90, or 180 mg/kg via intravenous drip infusion over 3, 6, or 12 minutes, respectively, on Days 15, 22, and 29; and (iii) at 45 mg/kg via bolus intravenous injection on Day 46.

During the study period, there were no toxicological findings in clinical signs or body weight except for high levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

3.(iii).A.(2) Repeat-dose toxicity
3.(iii).A.(2).1) Four-week repeated intravenous administration maximum tolerable dose finding toxicity study in rats (4.2.3.2.1: reference data)
Asfotase alfa was intravenously administered to male and female juvenile rats at 10, 30, 90, or 180 mg/kg/week once weekly for 4 weeks.

Swelling and skin discoloration of the limbs, auricles, and muzzle were observed in the 10 and 30 mg/kg groups following the second and subsequent doses. Decreased motility, piloerection, hyperpnea, and the swelling and skin discoloration of the limbs, auricles, and muzzle were observed in the ≥90 mg/kg/week groups following the first and subsequent doses, and the erosion and ulceration of glandular stomach were detected at necropsy and on histopathological examination.

On the basis of the above results and serious clinical symptoms observed in the 180 mg/kg/week group, use of 180 mg/kg/week as the maximum dose in a longer-term study was considered inappropriate.

3.(iii).A.(2).2) Four-week repeated intravenous administration toxicity study in rats (4.2.3.2.2)
Asfotase alfa was intravenously administered to male and female juvenile SD rats at 0 (vehicle[15]), 2.6, 26, or 77 mg/kg/week once weekly for 4 weeks. The study also had a recovery test group, in which the reversibility of toxicity was evaluated after a 4-week drug withdrawal period.

After administration, acute reactions (e.g., half-closed eye, decreased motility, rounded posture, decreased body temperature, and discoloration and swelling of the forelimb and hind footpad) occurred in the asfotase alfa groups, and high serum phosphorus levels were observed in male rats in the 77 mg/kg/week group.

In the asfotase alfa groups, a dose-dependent reduction in body weight gain, though minor, was observed in male rats during the recovery period, and decreased bone area and bone mineral content were observed in both male and female rats at the end of the recovery period.

Taking account of serious acute reactions, reduced body weight gain, and bone growth suppression observed in the 77 mg/kg/week group, the no-observed-adverse-effect level (NOAEL) was determined to be 26 mg/kg/week.

3.(iii).A.(2).3) Twenty six-week repeated intravenous administration toxicity study in rats (4.2.3.2.3)
Asfotase alfa was intravenously administered to male and female juvenile SD rats at 0 (vehicle[15]), 1, 3, or 13 mg/kg/day once daily for 26 weeks. The study also had a recovery test group, in which the reversibility of toxicity was evaluated after a 4-week drug withdrawal period. Two rats in the control group and 2 rats in the 1 mg/kg/day group died during the administration period due to unknown causes.

While acute reactions (e.g., swelling and redness of skin) occurred after administration in the asfotase alfa groups, no toxicological signs were detected in body weight, food consumption, ophthalmologic examination, hematology, biochemistry, urinalysis, bone metabolism markers (osteocalcin, C-telopeptide), bone density, bone geometry, behavior observation, physical growth, estrous cyclicity, sperm test, organ weight measurement, necropsy, or histopathological examination.

Other than transient acute reactions observed in the asfotase alfa groups, no toxicological signs were detected by any of the above examinations. Therefore, the NOAEL was determined to be 13 mg/kg/day.

The exposure to asfotase alfa in serum ($AUC_{0-168\,h\,ss}$) at Week 26 of administration at 13 mg/kg/day was 1,610,000 U·h/L, which was approximately 3.9-fold higher than the exposure after administration at the clinical dose.[34]

3.(iii).A.(2).4) Four-week repeated intravenous administration toxicity study in monkeys (4.2.3.2.4)
Asfotase alfa was intravenously administered to male and female juvenile cynomolgus monkeys at 0 (vehicle[15]), 5, 15, or 45 mg/kg/week once weekly for 4 weeks. The study also had a recovery test group, in which the reversibility of toxicity was evaluated after a 4-week drug withdrawal period.

No toxicological signs were detected in clinical condition, body weight, food consumption, ophthalmologic examination, ECG, hematology, biochemistry, urinalysis, bone metabolism markers (osteocalcin, C-telopeptide), bone density, physical examination (head circumference and the lengths of the upper posterior arm, forearm, thigh, and lower leg), organ weight measurement, necropsy, or histopathological examination.

Based on the above, the NOAEL was determined to be 45 mg/kg/week.

3.(iii).A.(2).5) Twenty six-week repeated subcutaneous administration toxicity study in monkeys (4.2.3.2.5)

Asfotase alfa was subcutaneously administered to male and female juvenile cynomolgus monkeys at 0 (vehicle[15]), 0.43, 2.14, or 10 mg/kg/day once daily for 26 weeks. The study also had a recovery test group, in which the reversibility of toxicity was evaluated after a 4-week drug withdrawal.

In the asfotase alfa groups, scab, dryness, and redness were observed on the skin at the injection site, and granulomatous inflammation was found on histopathological examination. No toxicological signs were detected in body weight, food consumption, ophthalmologic examination, ECG, hematology, biochemistry, bone metabolism markers (osteocalcin, type I procollagen, N-terminal propeptide, C-telopeptide, and N-telopeptide), urinalysis, bone density, bone geometry, organ weight measurement, necropsy, or histopathological examination.

While granulomatous inflammation was observed on the skin at the injection site, asfotase alfa was well tolerated in monkeys. On the basis of these results, the NOAEL was determined to be 10 mg/kg/day. The exposure to asfotase alfa in serum ($AUC_{0-168\,h\,ss}$) at Week 26 of administration at 10 mg/kg/day was 6710 U·h/L, which was approximately 2.5-fold higher than the exposure after administration at the clinical dose.[34]

3.(iii).A.(3) Genotoxicity

Asfotase alfa, a large protein molecule, does not permeate cell membranes or nuclear membranes, and is unlikely to bind to DNA or other chromosomal materials in cells. Therefore, no genotoxicity studies have been conducted.

3.(iii).A.(4) Carcinogenicity

No carcinogenicity studies have been conducted. Given that asfotase alfa has not been reported to have an anabolic effect and that its binding to HA does not induce signal transmission, it is considered that asfotase alfa is unlikely to be carcinogenic.

3.(iii).A.(5) Reproductive and developmental toxicity
3.(ii).A.(5).1) Rat studies of fertility and early embryonic development to implantation (4.2.3.5.1.1, 4.2.3.5.1.2)

In the dose-finding study, asfotase alfa was intravenously administered to male SD rats at 0 (vehicle[15]), 25, or 50 mg/kg/day once daily for 15 days. Acute reactions (abnormal gait, decreased motility, skin redness and swelling of the muzzle and limbs) and reduced body weight gain occurred in the asfotase alfa groups, and decreased food consumption was observed in the 50 mg/kg/day group.

In the main study, asfotase alfa was intravenously administered to male and female SD rats at 0 (vehicle[15]), 10, 25, or 50 mg/kg/day once daily from 28 days prior to mating through the mating period until the day of necropsy for males and from 14 days prior to mating through the mating period until 7 days after mating for females. Rats were mated within their respective dose groups.

The following changes in clinical condition were observed in parent animals: acute reactions (e.g., decreased activity, skin discoloration and swelling of limbs and muzzle) in the asfotase alfa groups and reduced body weight gain in male animals of the 50 mg/kg/day group. There were no effects of asfotase alfa on the reproductive function of males (sperm count, sperm motility, sperm morphology), that of females (estrous cyclicity, number of days required for mating, copulation ratio, pregnancy ratio, conception ratio, number of corpus luteum, number of implantation sites, pre- and post-implantation loss), or early embryonic development (number of live and dead embryos, number of resorptions).

Based on the above results, the NOAEL was determined to be 25 mg/kg/day for general toxicity in male animals, and 50 mg/kg/day for general toxicity in female animals and fertility and early embryonic development in male and female animals.

3.(ii).A.(5).2) Embryo-fetal development studies in rats (4.2.3.5.2.1, 4.2.3.5.2.2)
In the dose-finding study, asfotase alfa was intravenously administered to pregnant SD rats at 0 (vehicle[15]), 13, 25, or 50 mg/kg/day once daily from gestation day 6 to 19. The following changes were observed in parent animals: acute reactions (skin redness and swelling of the muzzle and limbs) in the asfotase alfa groups and half-closed eyes and decreased food consumption in the 50 mg/kg/day group. There were no effects on embryo-fetal development.

In the main study, asfotase alfa was intravenously administered to pregnant SD rats at 0 (vehicle[15]), 13, 25, or 50 mg/kg/day once daily from gestation day 6 to 19.

The following changes were observed in maternal animals: skin discoloration or swelling of limbs, auricles, and muzzle in the asfotase alfa groups and abnormal behaviors (e.g., decreased activity, incoordination, half-closed eyes) in the ≥25 mg/kg/day groups. There were no effects on the reproductive function (e.g., number of implantation sites, post-implantation loss) of maternal animals or on embryo-fetal development (fetal weight, fetal morphology, visceral and skeletal condition).

On the basis of the above results and taking account of the abnormal behaviors observed in the ≥25 mg/kg/day groups, the NOAEL was determined to be 13 mg/kg/day for general toxicity in maternal animals and 50 mg/kg/day for embryo-fetal development.

3.(ii).A.(5).3) Embryo-fetal development studies in rabbits (4.2.3.5.2.3, 4.2.3.5.2.4)
In the dose-finding study, asfotase alfa was intravenously administered to pregnant NZW rabbits at 0 (vehicle[15]), 6, 13, 25, or 50 mg/kg/day once daily from gestation day 7 to 19. Reduced body weight gain was observed in the ≥25 mg/kg/day groups as an effect of treatment on maternal animals, and low fetal weight in the asfotase alfa groups and a large number of early and late resorptions in the ≥25 mg/kg/day groups were observed as effects on embryo-fetal development. However, all these changes were within the laboratory's historical range.

In the main study, asfotase alfa was intravenously administered to pregnant NZW rabbits at 0 (vehicle[15]), 10, 25, or 50 mg/kg/day once daily from gestation day 7 to 19.

The following effects of treatment were observed on maternal animals: pale discoloration of the kidney found at necropsy and moderate renal tubular mineralization in the renal cortex found on histopathological examination in 2 of 20 rabbits in the 50 mg/kg/day group. In rabbits, an excessive amount of calcium from the diet is absorbed into the body and a large quantity of it is excreted via the kidneys. As a result, local mineralization is often observed in the kidneys as a histopathological change. A slight change in calcium excretion may affect renal calcium homeostasis and thereby lead to metastatic mineralization. Although rabbits have the above-stated physiological characteristics, a causal relationship to asfotase alfa could not be ruled out for the renal tubular mineralization found in the study. There were no effects of the drug on reproductive function (e.g., number of implantation sites, post-implantation loss) of maternal animals or on embryo-fetal development (number of live fetuses, fetal weight, fetal morphology, visceral and skeletal condition).

Based on the above results and taking account that a causal relationship to asfotase alfa could not be ruled out for the renal tubular mineralization observed in maternal animals in the 50 mg/kg/day group, the NOAEL was determined to be 25 mg/kg/day for general toxicity in maternal animals and 50 mg/kg/day for embryo-fetal development.

3.(ii).A.(5).4) Rat study for effects on pre- and post-natal development, including maternal function (4.2.3.5.3.1)
Asfotase alfa was intravenously administered to pregnant SD rats at 0 (vehicle[15]), 10, 25, or 50 mg/kg/day once daily from gestation day 6 to lactation day 21.

In maternal parents, acute reactions (e.g., skin discoloration and swelling of limbs, auricles, and muzzle) were observed in the asfotase alfa groups. Cannibalism was also observed, and the number of relevant cases in the 25 mg/kg/day group was higher than the laboratory's historical range. While its relationship

to asfotase alfa is unclear, it cannot be ruled out that cannibalism may have been an acute reaction to the treatment.

Although reduced body weight gain and low food consumption were observed in post-weaning male F_1 pups in the 50 mg/kg/day group, these changes were too minor to be regarded as toxic. There were no effects of asfotase alfa on F_1 pups in terms of survival, physical growth, or reproductive function, or on F_2 pups.

On the basis of the above results, the NOAEL was determined to be 50 mg/kg/day for both maternal animals and F_1 pups.

3.(ii).A.(6) Local tolerance
Local tolerance study in rats (4.2.3.6.1)
Asfotase alfa was subcutaneously administered to juvenile SD rats at 0 (vehicle[15]), 0.84, 8.4, or 25.2 mg/kg/day once daily for 28 days.

Mononuclear cell infiltration at the injection site occurred in the asfotase alfa groups, and swelling of axillary lymph nodes and lymphoid hyperplasia were observed in the ≥8.4 mg/kg/day groups. However, asfotase alfa was well tolerated in rats.

3.(iii).B   Outline of the review by PMDA
PMDA considers that the submitted data reveal no specific toxicological problems and that there are no toxicologically significant findings from the toxicity studies of asfotase alfa. Although acute reactions were observed in rats following intravenous administration of asfotase alfa, the applicant regarded them as toxicologically insignificant because no such findings were detected following subcutaneous administration to rats, intravenous administration to rabbits, or intravenous or subcutaneous administration to cynomolgus monkeys. From a toxicological viewpoint, PMDA accepts the explanation of the applicant. However, in the section for clinical data, further review should be undertaken to investigate the effect of asfotase alfa on humans [see "4.(iii).B.(3).2) Injection-related reactions"].

4. Clinical data
4.(i)   Summary of biopharmaceutic studies and associated analytical methods
4.(i).A   Summary of the submitted data
When the enzyme activity of asfotase alfa in human serum was quantified by an enzyme activity assay using *p*-nitrophenyl phosphate as substrate, the lower limit of quantification[49] was 5, 10, or 150 ng/mL.[50] Anti-asfotase alfa-antibodies in serum were detected by an electrochemiluminescence

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[49] The lower limit of quantification was changed because both the analysis laboratory and the procedures were changed. The comparability of respective analysis laboratories was confirmed by cross validation.

[50] Enzyme activity (U/L) = enzyme concentration (ng/mL) × specific activity (U/mg) × (1 mg/1,000,000 ng) × (1000 mL/L); One unit (U) of enzyme activity was defined as the amount of enzyme with which *p*-nitrophenyl phosphate (pNPP) was degraded to produce 1 μmol of *p*-nitrophenol (pNP) in 1 minute.

immunoassay, and neutralizing antibodies were detected by an enzyme activity assay using *p*-nitrophenyl phosphate as substrate. During the clinical development of asfotase alfa, the batch size was changed from ▮ L to ▮ L, the same size as that of the proposed drug product. The formulation prepared with ▮ L batch was used in Study ENB-001-08 and the pre-change and post-change formulations were used in Studies ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10. The comparability of the 2 formulations has been confirmed [see "2.A.(1).4) Manufacturing process development (comparability)"].

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

As evaluation data, the results of a clinical pharmacology study in foreign patients with hypophosphatasia (5.3.5.2.1: Study ENB-001-08) were submitted. In addition, the results of a population pharmacokinetic analysis (5.3.4.2) performed using data from clinical studies (Studies ENB-001-08, ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10) were also submitted. The main results from the studies are described below.

4.(ii).A.(1) Patients

Clinical pharmacology study in foreign patients with hypophosphatasia (5.3.5.2.1: ENB-001-08 [▮▮▮ 20▮ to ▮▮▮ 20▮])

The safety, pharmacokinetics, and pharmacodynamic effects of asfotase alfa were investigated in an open-label, uncontrolled, dose-escalation study enrolling foreign patients with hypophosphatasia[51] (target number of subjects, 6; 1 subject of infantile-onset type, 3 subjects of childhood-onset type, and 2 subjects with unknown time of onset).

In both Cohort 1 and Cohort 2, asfotase alfa was administered at 3 mg/kg as a single intravenous dose in Week 1, and at 1 or 2 mg/kg once weekly as multiple subcutaneous doses from Week 2 to Week 4.

All of the 6 treated subjects (3 subjects in each cohort) were included in the pharmacokinetic analysis population, pharmacodynamic analysis population, and safety analysis population.

The pharmacokinetic parameters during the administration period are shown in Table 7.

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[51] Key inclusion criteria: patients aged ≥18 to ≤80 years with the following conditions
  • a serum ALP level that is lower than age-adjusted mean serum ALP level by ≥3 SD
  • the following X-ray findings found in hypophosphatasia: osteopenia or osteomalacia
  • at least 2 of the following findings related to hypophosphatasia: plasma PLP level higher than mean plasma PLP level by ≥2.5 SD; current or previous non-traumatic fracture, pseudofracture, or delayed fracture healing; a history of rickets; a history of premature exfoliation of primary teeth; bone mutation corresponding to a history of osteomalacia or rickets

Table 7. Pharmacokinetic parameters during the administration period of asfotase alfa

	Cohort 1 (n = 3)			Cohort 2 (n = 3)		
Mode of administration	IV	SC		IV	SC	
Time point	Week 1	Week 2	Week 4	Week 1	Week 2	Week 4
C_{max} (U/L)	42,694 ± 8443	514 ± 119	340 ± 206	46,890 ± 6635	1081 ± 65.2	1020 ± 326
$AUC_{0-\infty}$ (U·h/L)	265,798 ± 95,160	–	–	368,978 ± 16,615	–	–
AUC_{τ} (U·h/L)	232,571 ± 88,022	66,034 ± 19,241	40,444[a]	327,737 ± 15,937	138,595±6958	136,109 ± 41,875
T_{max} (h)	1.25	24.2	35.9	1.50	48.0	48.1
$T_{1/2}$ (h)	72.8 ± 8.75	112.9, 110.8[b]	120[a]	58.8 ± 7.88	97.6, 121.6[b]	135 ± 27.8
CL (mL/min/kg)	11.4 ± 2.23	–	–	7.90 ± 1.79	–	–
Vz (L/kg)	71.0 ± 12.6	–	–	39.7 ± 8.11	–	–

Mean ± standard error except for T_{max} (median); –, not calculated;
C_{max}, maximum plasma concentration; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time zero to infinity (AUC extrapolated to infinity); AUC_{τ}, area under the plasma concentration-time curve during dosing interval;
T_{max}, time to maximum plasma concentration; $T_{1/2}$, elimination half-life; CL, total clearance; Vz, volume of distribution;
IV, intravenous administration; SC, subcutaneous administration
a) n = 1
b) n = 2 (individual values are shown)

The absolute bioavailability[52] (BA; calculated for individual subjects at Weeks 2 and 4) ranged from 61.6% to 98.4% in Cohort 1 and from 45.8% to 71.3% in Cohort 2.

In the analysis of pharmacodynamic effects, the plasma concentrations of inorganic pyrophosphate (PPi[53]) were between 5.87 and 6.86 μM at baseline and between 3.01 and 4.43 μM at Week 4 after subcutaneous administration.[54] The plasma concentrations of pyridoxal 5'-phosphate (PLP[55]) in Cohort 1 were 44.1 or >100 μM at baseline and 8.5 to 84.0 μM at Week 4 after subcutaneous administration, and those in Cohort 2 were >100 μM at baseline and 27.2, 43.7, and >100 μM at Week 4 after subcutaneous administration.

In the safety analysis, 9 adverse events were reported in 2 of 3 subjects in Cohort 1, and 41 adverse events in 3 of 3 subjects in Cohort 2. Of these, events for which a causal relationship to the study drug could not be ruled out (hereinafter, "adverse drug reactions") were 3 events (headache, chills, nausea) in 1 subject in Cohort 1 and 26 events (somnolence and headache [3 events each], chills, cough, feeling hot, paraesthesia, flushing, and musculoskeletal pain [2 events each], and nausea, blood pressure decreased, injection site pain, infusion site pain, feeling abnormal, pain in extremity, light headedness, and epigastric pain [1 event each]) in 2 subjects in Cohort 2.

There were no deaths, serious adverse events, or adverse events leading to study discontinuation.

Two subjects were positive for anti-asfotase alfa-antibodies while no subjects developed neutralizing antibodies.

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[52] The absolute BA was calculated based on dose-normalized $AUC_{0-\infty}$ (for intravenous administration) and $AUC_{0-168 h}$ (for subcutaneous administration).

[53] ███

[54] Plasma PPi concentrations in Cohort 1 were not measured because samples obtained from the cohort were not spiked with any TNSALP inhibitor.

[55] ███

4.(ii).A.(2) Other analysis

Population pharmacokinetic (PPK) analysis (5.3.4.2)

Population pharmacokinetic (PPK) analysis was performed on the serum asfotase alfa concentration data from samples collected at 1183 time points [56] from clinical studies in patients with hypophosphatasia (Studies ENB-001-08, ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10), using a non-linear mixed effect model. A two-compartment model with first-order absorption was used as the basic model (software used, NONMEM ver. 7.2).

The PPK analysis population included 60 subjects (29 males and 31 females) with the following baseline characteristics (mean [minimum, maximum]): age, 17.4 (0.00406, 66.8); body weight, 32.3 (2.21, 90.7) kg; ALT, 28.9 (6.00, 484) U/L; AST, 37.6 (16.0, 415) U/L; serum creatinine, 46.4 (17.0, 106) mg/dL; and total sialic acid, ▮ (▮, ▮) mol/mol. Candidate covariates for clearance (CL) included age, body weight, presence or absence of anti-asfotase alfa antibodies and neutralizing antibodies, total sialic acid content in a formulation batch, estimated glomerular filtration rate (eGFR), ALT, AST, and serum creatinine. Relative bioavailability between 2 batch sizes (▮ and ▮ L) was also included in candidate covariates. Significant covariates for CL selected from these candidate covariates were body weight, immunogenicity (presence or absence of anti-asfotase alfa antibodies and neutralizing antibodies), and total sialic acid content. The following effects of covariates were estimated: the CL in patients with a body weight of 2.89 to 72.2 kg varied within a range from 1.43 to 23.6 L/day; the CL in patients who were positive for anti-asfotase alfa antibodies and negative for neutralizing antibodies or those who were positive for both anti-asfotase alfa antibodies and neutralizing antibodies was increased by 9% to 20% compared with the CL in patients who were negative for both anti-asfotase alfa antibodies and neutralizing antibodies; and the CL decreased as total sialic acid content increased.[57] The estimated effect of batch size was as follows: The relative BA of the batch size of ▮ L to that of ▮ L and its 90% confidence interval were 86.9% [80.0%, 94.3%].

4.(ii).B   Outline of the review by PMDA

4.(ii).B.(1) Pharmacokinetics in Japanese and foreign patients

PMDA asked the applicant to explain the pharmacokinetics in Japanese and foreign patients.

The applicant responded as follows:

In the global clinical trial in patients with infantile-onset hypophosphatasia including Japanese patients (Study ENB-010-10), the pharmacokinetics after administration of asfotase alfa at 2 mg/kg was compared between Japanese and foreign subjects. The mean serum asfotase alfa concentration (minimum, maximum) at 6, 24, 32, and 48 hours after the first dose was 863 (444, 1267), 954 (568, 1587), 1065 (individual subjects' values [n = 2]: 727, 1402), and 1052 (668, 1555) U/L, respectively, in

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[56] PPK analysis was performed on data available by ▮ 20▮. The data were from 135 samples obtained after intravenous administration and 1048 samples obtained after subcutaneous administration, and analyzed by 2 different methods.

[57] Steady-state serum asfotase alfa concentrations following subcutaneous administration of asfotase alfa at 2 mg/kg 3 times weekly were simulated. The results suggested that the mean steady-state serum asfotase alfa concentration ranged from 967 to 3149 U/L with an increase in total sialic acid content from ▮ to ▮ mol/mol.

Japanese subjects (n = 2-4); and 649 (170, 2418), 881 (259, 3130), 870 (158, 3371), and 678 (233, 1848) U/L, respectively, in foreign subjects (n = 16-17). These concentrations varied widely but no marked difference between the 2 patient populations was evident. Through the PPK analysis and pharmacokinetic/pharmacodynamic (PK/PD) analysis[58] of data from 60 foreign subjects in clinical studies in hypophosphatasia patients (Studies ENB-001-08, ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10), the 90% prediction intervals for serum asfotase alfa concentration were calculated. A visual comparison was made by overlapping these values with the measured values of serum asfotase alfa concentrations and each clinical endpoint in 5 Japanese subjects enrolled in Study ENB-010-10. The serum concentrations of asfotase alfa in 2 Japanese subjects at some time points were higher than the 90% prediction intervals of a PPK analysis model of serum asfotase alfa concentration in foreign subjects, and were higher than the measured values of serum asfotase alfa concentrations in foreign subjects. This result may be partially attributable to the high total sialic acid content and specific activity of the formulation administered to the 2 Japanese subjects [see "4.(ii).B.(3) Total sialic acid content in asfotase alfa"]. However, the serum concentrations of asfotase alfa in these subjects measured at the other time points and those in the other 3 Japanese subjects were within the range of 90% prediction intervals of a PPK analysis model of serum asfotase alfa concentration in foreign subjects within the range of the measured values of serum asfotase alfa concentrations in foreign subjects. A similar result was obtained when the values of individual clinical endpoints in Japanese subjects were compared with measured values in foreign subjects and the 90% prediction intervals of a PK/PD analysis model in foreign subjects. No marked difference was evident between the 2 patient populations. Furthermore, asfotase alfa is a protein formulation, and thus, its metabolism is unlikely to be affected by differences in ethnicity. While it is difficult to make a definite conclusion due to the very limited number of Japanese patients enrolled in the clinical study, pharmacokinetics in Japanese patients is unlikely to be notably different from that in foreign patients.

PMDA considers as follows:

Rigorous evaluation of pharmacokinetics in Japanese and foreign patients is difficult because it is unclear why serum concentrations of asfotase alfa in some Japanese subjects were higher than those in foreign subjects in the global clinical study and because of the very limited number of Japanese patients who were enrolled in the study. However, taking into account the pharmacokinetic properties of asfotase alfa, the applicant's response that there is no marked difference in pharmacokinetics between Japanese and foreign patients is acceptable.

4.(ii).B.(2) Immunogenicity of asfotase alfa
The applicant provided the following explanation:
The effect of antibodies on the pharmacokinetics of asfotase alfa was investigated by conducting a PPK analysis using the serum asfotase alfa concentration data of 582 samples from 58 subjects undergoing antibody measurement out of the subject data obtained from clinical studies in hypophosphatasia

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[58] E_{max} model based PK/PD analysis was performed for plasma PPi concentrations and RGI-C scores and RCC scores obtained from the X-ray images used for the assessment of changes in the severity of rickets (software used, NONMEM ver. 7.2).

patients (Studies ENB-001-08, ENB-002-08/003-08, ENB-006-09/008-10, ENB-009-10, and ENB-010-10). The data analyzed show that 45 of 58 subjects (78%) were positive for anti-asfotase alfa-antibodies at least at 1 time point and that, of these 45 subjects, 13 were positive and 32 were negative for neutralizing antibodies. The titer of anti-asfotase alfa-antibodies (median [minimum, maximum]) was 4 (0, 2048) units. Based on the results of the PPK analysis[59], the CL (estimate [90% confidence interval]) was estimated to be 9% [4%, 13%] higher in patients who were positive for anti-asfotase alfa-antibodies and negative for neutralizing antibodies and 20% [7%, 34%] higher in those who were positive for both anti-asfotase alfa-antibodies and neutralizing antibodies than in patients who were negative for both anti-asfotase alfa-antibodies and neutralizing antibodies. The steady-state serum concentration (estimated mean concentration during dosing interval [90% confidence interval]) of asfotase alfa was estimated to be 2251 [1918, 2647] U/L in patients who were negative for both anti-asfotase alfa-antibodies and neutralizing antibodies; 2069 [1760, 2433] U/L in those who were positive for anti-asfotase alfa-antibodies and negative for neutralizing antibodies; and 1883 [1592, 2232] U/L in those who were positive for both anti-asfotase alfa-antibodies and neutralizing antibodies. Given that the 90% confidence intervals for the estimated steady-state serum concentrations of asfotase alfa included overlapping values shared by the respective immunogenicity profiles, it is unlikely that immunogenicity will have any clinically relevant effect on the efficacy of asfotase alfa.

PMDA asked the applicant to explain the efficacy and safety of asfotase alfa in subjects with high titers of anti-asfotase alfa-antibodies in clinical studies.

The applicant responded as follows:
High titers of anti-asfotase alfa-antibodies were observed in 4 subjects (1 in Study ENB-001-08 and 3 in Study ENB-006-09), and the titers in these subjects exceeded 500 by 60 to 120 weeks after administration. As efficacy data from these subjects, the radiographic global impression of change[60] (RGI-C) score at the final assessment was 2.66, 0.67, 2.67, and 3.00, and the rickets severity scale[61] (RSS) score was 0.0, 1.0, 0.0, and 0.0, showing no obvious difference from the scores in the overall subject population. As safety data from these 4 subjects, 10, 52, 35, and 23 adverse events were reported, including serious adverse events (respiratory distress, lower respiratory tract infection, central venous

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[59] Steady-state serum asfotase alfa concentrations after subcutaneous administration at 2 mg/kg 3 times weekly were estimated on the assumption that the covariates of body weight, total sialic acid content, and batch size were 22.7 kg, ■■ mol/mol, and ■■ L, respectively.

[60] Three independent and highly-trained radiologists examined baseline X-ray findings, and then evaluated X-ray images of the chest, wrists, or knee joints taken before and after the start of treatment to assess rickets-related changes from baseline using RGI-C score, while being blinded, after baseline, to subject information including time points for taking post-baseline X-ray images. Growth plate abnormalities associated with rickets and bone morphology were assessed and rated on the following 7-point scale: −3 (severe worsening of rickets), −2 (moderate worsening of rickets), −1 (slight worsening of rickets), 0 (no change), 1 (slight improvement of rickets), 2 (substantial improvement of rickets), and 3 (complete or near-complete healing of rickets).

[61] One independent specialist who was blinded to any subject information including evaluation time points read and assessed all subjects' X-ray images of the wrists and knee joints taken over time before and after the start of treatment. Assessment was performed using the rickets severity scale (RSS; the maximum score for the wrist, 4; the maximum score for the knee joint, 6; severe rickets, 10; absence of rickets, 0). The RSS was developed to evaluate the severity of rickets affecting the development of wrists and knee joints on the basis of the degree of fraying and cupping of the metaphyses and the percentage of deformity seen in the growth plates (Thacher TD, et al. *J Trop Pediatr.* 2000;46:132-9).

catheter removal, craniosynostosis, and scoliosis) reported in 1 subject. However, there was no obvious difference in safety data between these subjects and the overall subject population.

PMDA accepted the applicant's response. However, PMDA considers that the effect of immunogenicity on the efficacy and safety of asfotase alfa should be further discussed in the section below [see "4.(iii).B.(3).4) Effects of antibody production"].

4.(ii).B.(3) Total sialic acid content in asfotase alfa

The applicant provided the following explanations:

The relationship between the pharmacokinetics of asfotase alfa and the total sialic acid content after single-dose intravenous or subcutaneous administration was analyzed using the data obtained from pharmacokinetic studies in mice.[62] The results revealed a positive correlation between the total sialic acid content and dose-normalized $AUC_{0-\infty}$ ($R^2 = 0.395$, $P = 0.0040$) after single-dose intravenous administration, which suggests that a negative correlation could exist between the total sialic acid content and clearance ($R^2 = 0.448$, $P = 0.0017$). Also after single-dose subcutaneous administration, a positive correlation with the total sialic acid content was suggested for BA[52] ($R^2 = 0.675$, $P < 0.0001$) and dose-normalized C_{max} ($R^2 = 0.514$, $P = 0.0005$). A two-dimensional plot showed no correlation between the total sialic acid content and any of the following enzyme activity parameters: relative activity, hydroxyapatite (HA)-binding activity, Michaelis constant, and molecular activity. From the PPK analysis,[63] the steady-state blood asfotase alfa concentrations (estimated mean concentrations during dosing interval) for various total sialic acid contents (■, ■, ■, ■, ■, ■, ■, ■, and ■ mol/mol)[64] were estimated to be 967, 1494, 1709, 1926, 2145, 2365, 2587, 2923, and 3149 U/L, respectively.

The sialic acid content of a protein is known to affect its pharmacokinetics because of the following reasons: in comparison with glycoproteins with a high sialic acid content, glycoproteins with a low sialic acid content are less likely to be transferred from the negatively charged extracellular matrix into lymphatic vessels; glycoproteins with a low sialic acid content bind to hepatic asialoglycoprotein receptors, leading to relatively rapid receptor-mediated clearance in the liver; and decreased interaction of the glycoproteins with Fc receptors results in an increased susceptibility to protein degradation.[65] This is true of asfotase alfa, and its total sialic acid content affects the absorption, distribution, and excretion of the protein preparation. The subcutaneous administration of asfotase alfa with a low sialic acid content would promote its clearance, leading to a decrease in the AUC of asfotase alfa, which results in its reduced absorption, thereby decreasing its BA and C_{max}.

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[62] ALP-PC-22, 23, 24, 28, 29, 31, 37, 38, 40, 42, 43, 45, 46, 47, 58, 59, 60, 61, 62, 64, 67, 68, 69, 70, and 71 studies

[63] Steady-state serum asfotase alfa concentrations after subcutaneous administration at 2 mg/kg 3 times weekly were estimated on the assumption that the covariates of body weight, anti-asfotase alfa-antibody status, and a batch size were 22.7 kg, negative, and ■ L, respectively.

[64] Specification for total sialic acid content in the formulation, ■ to ■ mol/mol

[65] Bork K, et al. *J Pharm Sci*. 2009;98:3499-508; Liu L, et al. *Pharm Res*. 2013;30:803-12; Richter WF, et al. *AAPS J*. 2012;14:559-70

PMDA asked the applicant to explain the safety and efficacy of formulations with a higher sialic acid content within the range of the proposed specification.

The applicant responded as follows:

In clinical studies, subjects received formulations with different sialic acid content, and a rigorous comparison is therefore difficult. A total of 14 subjects (5 in Study ENB-006-09 and 9 in Study ENB-009-10) received formulations with a higher sialic acid content (■ to ■ mol/mol) at some time during the study period. Some efficacy outcomes[66] differed between the subjects who received formulations with a higher sialic acid content ("subgroup A") and those who received any of the other formulations ("subgroup B"); in the subgroups A and B, the change in plasma PPi concentration from baseline to Week 24, the primary endpoint in Study ENB-009-10, was -2.31 ± 1.3 μM and -1.63 ± 1.15 μM, respectively, and the change in plasma PLP concentration from baseline to Week 24 was -386 ± 441 ng/mL and -251 ± 387 ng/mL, respectively. However, the median change in 6-minute walk distance from baseline to Week 24 was 35.0 m and 29.0 m, respectively, showing no major difference between the two subgroups. Safety outcomes were similar between the two subgroups. In Study ENB-006-09/008-10, 200 adverse events occurred in 5 of 5 subjects in the subgroup A and 105 adverse events in 8 of 8 subjects in the subgroup B; 105 adverse drug reactions occurred in 5 of 5 subjects in the subgroup A and 124 adverse drug reactions in 7 of 8 subjects in the subgroup B; and no serious adverse events occurred in 5 subjects in the subgroup A or in 8 subjects in the subgroup B. In Study ENB-009-10, 340 adverse events occurred in 9 of 9 subjects in the subgroup A and 305 adverse events in 10 of 10 subjects in the subgroup B. In this study, 114 adverse drug reactions occurred in 7 of 9 subjects in the subgroup A and 171 adverse drug reactions in 7 of 10 subjects in the subgroup B; and 4 serious adverse events occurred in 3 of 9 subjects in the subgroup A and 2 serious adverse events in 1 of 10 subjects in the subgroup B. In clinical studies, no subjects received any formulations with a sialic acid content higher than the proposed specification, and the safety and efficacy of formulations with a sialic acid content within the range of the proposed specification, including those with a higher sialic acid content, are considered to be of no major concern.

PMDA considers that the safety and efficacy of treatment only with formulations with a higher sialic acid content are difficult to assess with any precision. However, given that no subjects received formulations with a sialic acid content higher than the proposed specification in clinical studies, PMDA accepted the applicant's response that the safety and efficacy of formulations with a sialic acid content within the range of the proposed specification are of no major concern.

4.(iii)   Summary of clinical efficacy and safety
4.(iii).A   Summary of the submitted data
As the evaluation data, the results from the following studies were submitted: clinical pharmacology study in foreign patients with hypophosphatasia (Study ENB-001-08), global clinical study in patients with infantile hypophosphatasia including Japanese patients (Study ENB-010-10), clinical study in

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[66] No subjects received formulations with a high total sialic acid content during the 24-week treatment in Study ENB-006-09.

foreign patients with infantile hypophosphatasia (Study ENB-002-08/ENB-003-08), clinical study in foreign patients with infantile and childhood hypophosphatasia (Study ENB-006-09/ENB-008-10), clinical study in foreign patients with infantile, childhood, and adult hypophosphatasia (Study ENB-009-10), and study of the natural clinical course of patients with infantile hypophosphatasia (Study ENB-011-10). The major results of the studies are shown below.

4.(iii).A.(1) Clinical pharmacology study
For a summary of the clinical pharmacology study in foreign patients with hypophosphatasia (Study ENB-001-08), see "4.(ii) Summary of clinical pharmacology studies."

4.(iii).A.(2) Global clinical study in patients with infantile hypophosphatasia including Japanese patients (5.3.5.2.3, ENB-010-10 [ongoing since July 2010; data cut-off,[67] ▮▮▮▮▮▮, 20▮▮])
The efficacy and safety of asfotase alfa were investigated in an open-label, uncontrolled study enrolling Japanese and foreign[68] patients with infantile hypophosphatasia[69] who were aged ≤5 years and who had developed hypophosphatasia at age <6 months (target sample size of 60, including 5 Japanese subjects). The study is currently underway.[70]

Subjects received asfotase alfa subcutaneously either at 1.0 mg/kg 6 times weekly or at 2.0 mg/kg 3 times weekly. It was permitted to adjust the dose according to change in body weight or in response to the results of safety and efficacy evaluations.

All of the 28 subjects treated (including 5 Japanese subjects) were included in the safety analysis population and the full analysis set (FAS), which was defined as the primary efficacy analysis population. Three subjects discontinued the study. Two subjects died and 1 subject withdrew for personal reasons.[71]

In the efficacy analysis, the RGI-C score from baseline at Week 24 (median [minimum, maximum]), the primary endpoint, was 1.67 (-1.67, 3.00) for the entire subject population, demonstrating a statistically significant improvement in the severity of rickets compared to baseline severity ($P < 0.0001$; a two-sided significance level of 5%; LOCF; Wilcoxon signed-rank test).

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[67] The results at data cut-off on ▮▮▮, 20▮ were additionally submitted as the long-term safety data in Japanese subjects.

[68] The USA, Canada, Germany, Turkey, and Taiwan

[69] Key inclusion criteria: patients aged ≤5 years with the onset of symptoms of hypophosphatasia prior to 6 months of age and with the following conditions
• a serum ALP level that is below the lower limit of age-adjusted normal range
• a plasma PLP level that is above the upper limit of normal
• the following X-ray findings found in hypophosphatasia: flared and frayed metaphyses, severe generalized osteopenia, widened growth plates, and areas of radiolucency or sclerosis
• at least 2 of the following findings related to hypophosphatasia: history or presence of nontraumatic post-natal fracture or delayed fracture healing, history of nephrocalcinosis or elevated serum calcium, functional craniosynostosis, respiratory compromise or rachitic chest deformity, vitamin B_6 dependent seizures, and failure to thrive

[70] The study is planned to continue until asfotase alfa is approved and released to the market.

[71] After withdrawal from the study due to medical complications, the subject died of pneumonia.

Concerning the main secondary endpoints, the RGI-C scores from baseline at respective time points are shown in Table 8.

Table 8. RGI-C scores at respective time points (Study ENB-010-10, FAS)

		Week 12 (n = 27)	Week 24 (n = 26)	Week 48 (n = 15)	Week 72 (n = 12)	Week 96 (n = 11)	Week 120 (n = 10)
Median		1.00	1.83	2.00	2.17	2.67	2.50
(min, max)		(-1.67, 3.00)	(-0.33, 3.00)	(1.67, 3.00)	(1.00, 3.00)	(1.67, 3.00)	(1.67, 3.00)
Score category[a]	-3 to <-2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-2 to <-1	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-1 to <0	2 (7.4)	1 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	0 to <1	7 (25.9)	4 (15.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1 to <2	9 (33.3)	8 (30.8)	1 (6.7)	4 (33.3)	3 (27.3)	1 (10.0)
	2 to <3	5 (18.5)	9 (34.6)	11 (73.3)	5 (41.7)	3 (27.3)	6 (60.0)
	3	3 (11.1)	4 (15.4)	3 (20.0)	3 (25.0)	5 (45.5)	3 (30.0)

a) Score category, number of subjects (proportion [%])

The RSS score was rated by using radiographic findings different from those used for RGI-C score. The changes in the RSS score (median [minimum, maximum]) from baseline at Weeks 12, 24, 48, 72, 96, and 120 were -1.00 (-5.5, 3.5), -1.00 (-7.5, 4.0), -4.00 (-9.5, -0.5), -1.50 (-7.0, 1.0), -1.50 (-8.0, 1.5), and -1.50 (-8.5, 0.0), respectively.

Of the 10 subjects (including 5 Japanese subjects) who were on ventilation (tracheal cannulation, tracheotomy, or transnasal continuous positive airway pressure) at baseline, 2 subjects (including 1 Japanese subject) were completely weaned from respiratory assistance (one at Week 24 and the other at Week 36), and the treatment for 1 subject (Japanese) was changed to supplemental oxygen at Week 24. One of the 2 subjects on supplemental oxygen at baseline were weaned from all respiratory assistance from Week 3 to Week 72. Of the 16 subjects who were not on respiratory assistance at baseline, 12 remained unchanged throughout the study period.

The overall survival (number at risk, number of deaths, number censored) estimated by the Kaplan-Meier method at Weeks 24, 48, 96, and 168 was 96% (24, 1, 3), 91% (16, 2, 10), 84% (10, 3, 15), and 84% (1, 3, 24), respectively.

In analysis of growth, the number of standard deviations away from the mean of the normal distribution (hereinafter referred to Z score) of height and body weight were calculated. The normal distribution was adjusted by sex and age at each time point beforehand. The median Z score (minimum, maximum) at baseline, Week 24, Week 48, Week 72, Week 96, and Week 120 was -2.92 (-10.1, 0.2), -3.08 (-10.6, 0.3), -2.71 (-13.0, 0.1), -2.48 (-5.01, 0.2), -2.01 (-5.6, 0.4), and -2.27 (-6.8, 0.3), respectively, for height; and was -2.41 (-23.8, 0.0), -2.29 (-17.3, 0.0), -2.22 (-5.9, 0.0), -1.47 (-5.1, -0.4), -1.18 (-6.1, -0.1), and -1.24 (-4.5, 0.3), respectively, for body weight.

In analyses of biochemical markers, the plasma PPi concentration (mean ± standard deviation) at baseline and at Weeks 24, 48, and 96 was 6.82 ± 2.15, 3.65 ± 2.12, 4.39 ± 1.98, and 5.96 ± 6.96 μM, respectively, with the change from baseline being -3.11 ± 2.69 at Week 24, -2.21 ± 2.41 at Week 48, and

-0.26 ± 7.00 μM at Week 96. The plasma PLP concentration at baseline and at Weeks 24, 48, and 96 was 5083.3 ± 7130.6, 892.1 ± 3361.6, 396.3 ± 1233.8, and 162.3 ± 222.0 ng/mL, respectively, with the change from baseline being -2185.8 ± 4091.2 at Week 24, -1644.8 ± 2787.5 at Week 48, and -3916.6 ± 6039.6 ng/mL at Week 96.

Table 9 shows the subject characteristics and the results for the primary endpoint and main secondary endpoints in the Japanese subject population. The plasma PPi concentration at baseline and at Weeks 24 and 48 in this population was 6.38 ± 0.64, 2.51 ± 1.88, and 0.62 (n = 1) μM, respectively, with the change from baseline being -3.87 ± 1.77 at Week 24 and -5.46 μM at Week 48; and the plasma PLP concentration at baseline and at Week 24 was 9487.5 ± 7022.6 and 3720.3 ± 7319.8 ng/mL, respectively, with the change from baseline being -2575.5 ± 2583.3 ng/mL.

Table 9. Subject characteristics,[a] primary endpoints, and secondary endpoints
(Study ENB-010-10, Japanese subject population)

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5
Dose	2 mg/mL × 3/wk	2 mg/mL × 3/wk	2 mg/mL × 3/wk	2 mg/mL × 3/wk	2 mg/mL × 3/wk
Sex	■	■	■	■	■
Age (weeks)[a]	■	■	■	■	■
Final time point (Week)	48	24	24	36	24
Change from baseline in RGI-C score at Week 24	2.00	1.00	0.67	0.67	3.00
Change from baseline in RSS score at the final time point	-8.0	-2.0	0.0	2.5	-6.5
Respiratory function at baseline	Mechanical ventilation	Intermittent mandatory ventilation	Mechanical ventilation	Mechanical ventilation	Mechanical ventilation
Respiratory function at the final time point	Supplemental oxygen	Intermittent mandatory ventilation	Mechanical ventilation	Mechanical ventilation	Weaned from respiratory assistance
Change from baseline in Z-score for height at the final time point	-0.59	-0.19	-0.51	-0.78	0.98
Change from baseline in Z-score for body weight at the final time point	-2.47	-0.59	4.28	5.92	0.61

a) At baseline (Day 1); wk, week

In the safety analysis,[72] 796 adverse events were reported in 28 of 28 subjects (100%). Of these, 194 adverse events were those for which a causal relationship to the study drug could not be ruled out[73] (hereinafter, adverse drug reactions) found in 19 of 28 subjects (67.9%). The adverse events reported in ≥3 subjects and adverse drug reactions are shown in Table 10. In the Japanese population, 117 adverse events were reported in 5 subjects and 68 adverse drug reactions[74] in 3 subjects. The adverse events reported in ≥2 Japanese subjects and adverse drug reactions reported in ≥1 Japanese subject are shown in Table 11.

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[72] The total administration period in the safety analysis population was 39.62 person-years. The number of subjects by administration period was as follows: <3 months, 1; 3 to <6 months, 6; 6 to <9 months, 4; 9 to <12 months, 2; 12 to <24 months, 5; 24 to <36 months, 9; and ≥36 months, 1. The total administration period in Japanese subjects in the safety analysis population was 2.96 person-years. The analysis was made using data obtained up to Weeks 24, 36, and 48 from 3, 1, and 1 subject, respectively. Later, long-term safety data from Japanese subjects were additionally submitted. The additional data showed that the total administration period in Japanese subjects in the safety analysis population was 5.26 person-years, with an administration period of 48, 60, and 72 weeks for 3, 1, and 1 subject, respectively. The safety of asfotase alfa in the Japanese population in the study was based on the long-term data.

[73] Adverse events that were considered possibly, probably, or definitely related to the study drug

[74] Adverse events that were considered unlikely, possibly, probably, or definitely related to the study drug

Table 10. Adverse events reported in ≥3 subjects and adverse drug reactions
(Study ENB-010-10, entire subject population)

	Adverse event	Adverse drug reaction
Overall events	28 (100.0)	19 (67.9)
Injection site erythema	12 (42.9)	12 (42.9)
Pyrexia	12 (42.9)	1 (3.6)
Tooth loss	12 (42.9)	0 (0.0)
Vomiting	7 (25.0)	0 (0.0)
Constipation	7 (25.0)	0 (0.0)
Upper respiratory tract infection	7 (25.0)	0 (0.0)
Pneumonia	6 (21.4)	1 (3.6)
Diarrhoea	6 (21.4)	0 (0.0)
Injection site induration	5 (17.9)	5 (17.9)
Injection site discolouration	5 (17.9)	5 (17.9)
Nasopharyngitis	5 (17.9)	0 (0.0)
Respiratory disorder	5 (17.9)	0 (0.0)
Craniosynostosis	5 (17.9)	0 (0.0)
Feeding tube complication	4 (14.3)	0 (0.0)
Irritability	4 (14.3)	0 (0.0)
Rash	4 (14.3)	0 (0.0)
Papilloedema	4 (14.3)	0 (0.0)
Urinary tract infection	4 (14.3)	0 (0.0)
Injection site pain	3 (10.7)	3 (10.7)
Injection site haematoma	3 (10.7)	3 (10.7)
Injection site rash	3 (10.7)	3 (10.7)
Erythema	3 (10.7)	1 (3.6)
Contusion	3 (10.7)	1 (3.6)
Dyspnoea	3 (10.7)	0 (0.0)
Eczema	3 (10.7)	0 (0.0)
Oxygen saturation decreased	3 (10.7)	0 (0.0)
Cough	3 (10.7)	0 (0.0)
Bradycardia	3 (10.7)	0 (0.0)
Alanine aminotransferase increased	3 (10.7)	0 (0.0)
Food intolerance	3 (10.7)	0 (0.0)
Dermatitis diaper	3 (10.7)	0 (0.0)
Dry skin	3 (10.7)	0 (0.0)
Aspartate aminotransferase increased	3 (10.7)	0 (0.0)
Gastroenteritis	3 (10.7)	0 (0.0)
Respiratory distress	3 (10.7)	0 (0.0)
Hypercalcaemia	3 (10.7)	0 (0.0)
Intracranial pressure increased	3 (10.7)	0 (0.0)
Nephrocalcinosis	3 (10.7)	0 (0.0)
Hypertension	3 (10.7)	0 (0.0)

Adverse event, number of subjects with adverse events (incidence [%]); MedDRA/J ver. 13.0

Table 11. Adverse events reported in ≥2 subjects and adverse drug reactions reported in ≥1 subject
(Study ENB-010-10, Japanese subject population)

	Adverse event	Adverse drug reaction
Overall events	5 (100.0)	3 (60.0)
Injection site erythema	2 (40.0)	2 (40.0)
Pyrexia	2 (40.0)	1 (20.0)
Tooth loss	2 (40.0)	0 (0.0)
Nasopharyngitis	2 (40.0)	0 (0.0)
Injection site induration	1 (20.0)	1 (20.0)
Injection site swelling	1 (20.0)	1 (20.0)
Injection site pain	1 (20.0)	1 (20.0)
Hordeolum	1 (20.0)	1 (20.0)
Chills	1 (20.0)	1 (20.0)
Injection site pruritus	1 (20.0)	1 (20.0)

Number of subjects with adverse events or adverse drug reactions (incidence [%]); MedDRA/J ver. 13.0

In the entire subject population, 3 subjects died (respiratory failure and brain death, cardio-respiratory arrest, and pneumonia), and the pneumonia was assessed as an adverse drug reaction. No deaths occurred

in the Japanese subject population. In the entire subject population, there were no adverse events leading to study discontinuation other than the cardio-respiratory arrest reported in 1 of the 3 subjects who died. A total of 105 serious adverse events were reported in 17 of 28 subjects (60.7%) in the entire subject population. The serious adverse events reported in ≥2 subjects among this population were as follows: 6 episodes of respiratory disorder in 4 subjects; 4 episodes each of pneumonia, food intolerance, and pyrexia in 3 subjects each; 3 episodes of craniosynostosis in 3 subjects; 6 episodes of feeding tube complication in 2 subjects; and 2 episodes each of bronchiolitis, upper respiratory tract infection, viral infection, and irritability in 2 subjects each. In the Japanese subject population, the following 11 serious adverse events were reported in 4 of 5 subjects: 3 episodes of upper respiratory tract inflammation in 1 subject; and 1 episode each of pyrexia, chills, hydrocephalus, bronchitis, influenza, mechanical ventilation complication, performance of nuclear magnetic resonance imaging (MRI), and surgery of the respiratory tract in 1 subject each.

The following 180 episodes (including 61 episodes in 2 of 5 Japanese subjects[75]) of injection site reactions[76] were reported in 15 of 28 subjects (53.6%): 95 episodes of injection site erythema in 12 subjects (including 32 episodes in 2 Japanese subjects); 32 episodes of injection site induration in 5 subjects (including 22 episodes in 1 Japanese subject); 16 episodes of injection site discolouration in 5 subjects; 9 episodes of injection site pain in 3 subjects (including 3 episodes in 1 Japanese subject); 7 episodes of injection site haematoma in 3 subjects; 5 episodes of injection site swelling in 2 subjects (including 3 episodes in 1 Japanese subject); 2 episodes each of injection site rash and injection site reaction (PT) in 2 subjects each; 5 episodes of injection site urticarial in 1 subject; 4 episodes of injection site atrophy in 1 subject; 1 episode each of injection site haemorrhage, injection site macule, and injection site pruritus in 1 subject each (including 1 episode of injection site pruritus in 1 Japanese subject). These adverse events were either mild or moderate in severity. Four episodes (injection site rash; erythema; nausea and pyrexia) of injection-related reactions[77] were reported in 3 of 28 subjects (10.7%). They were all assessed as mild in severity except for the moderate nausea and pyrexia reported in 1 Japanese subject. Five episodes of ectopic calcification (3 episodes of nephrocalcinosis and 1 episode each of deposit eye and corneal deposits) were reported in 5 of 28 subjects (17.9%), but they were not identified as adverse drug reactions.

The serum calcium level (mean ± standard deviation) was 2.60 ± 0.39 mmol/L at baseline and 2.45 ± 0.28 to 2.61 ± 0.23 mmol/L from Week 3 to Week 168. The urine calcium/creatinine ratio (mean ± standard deviation) was 1.87 ± 1.73 mmol/mmol at baseline and 0.50 ± 0.35 to 1.94 ± 3.23 mmol/mmol from Week 3 to Week 168.

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[75] The long-term safety data in the Japanese subject population showed 63 injection site reactions in 2 of 5 subjects. They comprised 31 episodes of injection site erythema in 2 subjects, 24 episodes of injection site induration in 1 subject, 4 episodes of injection site swelling in 1 subject, 3 episodes of injection site pain in 1 subject, and 1 episode of injection site pruritus in 1 subject.

[76] An injection site reaction was defined as an adverse event that occurred at the injection site of the study drug at any time point after the start of study treatment and that was assessed as possibly, probably, or definitely related to the study drug.

[77] An injection-related reaction was defined as an adverse event exhibiting systemic sign(s), symptom(s), or finding(s) (e.g., urticaria generalised, pruritus, hypotension, respiratory distress) that occurred within 3 hours after administration of the study drug and that was considered possibly, probably, or definitely related to the study drug.

Of the 28 subjects evaluated, 22 (including 5 Japanese subjects) tested positive for anti-asfotase alfa antibodies with the maximum antibody titer being 1024. Of 28 the subjects, 14 (including 2 Japanese subjects) tested positive for neutralizing antibodies.[78]

4.(iii).A.(3) Clinical study in foreign patients with infantile hypophosphatasia (5.3.5.2.2, ENB-002-08/ENB-003-08 [ongoing since October 2008; data cut-off, ▉▉▉ 20▉])

The efficacy and safety of asfotase alfa were investigated in an open-label, uncontrolled study enrolling foreign patients with infantile hypophosphatasia who were aged ≤3 years and who had developed hypophosphatasia at age <6 months[79] (target sample size, 10). Study ENB-002-08 (24-week study) was followed by Study ENB-003-08, an ongoing extension study.[80]

Subjects received a single intravenous dose of asfotase alfa at 2.0 mg/kg, and then, after a 1-week run-in period, received multiple subcutaneous doses at 1 mg/kg 3 times weekly. After ≥1 month of subcutaneous administration, it was permitted to adjust the dose up to 40 mg/dose in response to the results of efficacy evaluation. The starting dose in Study ENB-003-08 was to be the same as the dose given at Week 24 in Study ENB-002-08.

All of the 11 subjects treated were included in the safety analysis population and the FAS, which was defined as the primary efficacy analysis population. One subject was withdrawn from the study due to injection-related reactions (piloerection, irritability, pyrexia, and chills) that occurred during the single-dose intravenous administration on Day 1. Of the 10 subjects who were enrolled in Study ENB-003-08 after completing Study ENB-002-08, 1 subject was withdrawn from the study due to septic shock.

In the efficacy analysis, the median RGI-C score (minimum, maximum) from baseline to Week 24, the primary endpoint, was 2.00 (0.00, 2.33), demonstrating statistically significant improvement in the severity of rickets compared to baseline ($P < 0.0039$; a two-sided significance level of 5%; LOCF; Wilcoxon signed-rank test).

Concerning the main secondary endpoints, the RGI-C scores over time from baseline to respective time points are shown in Table 12.

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[78] Assessed as positive when the *in vitro* enzyme activity inhibition rate exceeded 4.478%.

[79] Key inclusion criteria: patients aged ≤3 years with the onset of symptoms of hypophosphatasia prior to 6 months of age and with the following conditions
• a serum ALP level that is below the mean age-adjusted normal range by ≥3SD
• a plasma PLP level that is at least 4 times the upper limit of normal
• the following X-ray findings found in hypophosphatasia: flared and frayed metaphyses, severe generalized osteopenia, and widened growth plates
• at least 2 of the following findings related to hypophosphatasia: history or presence of nontraumatic post-natal fracture or delayed fracture healing, history of elevated serum calcium, functional craniosynostosis, nephrocalcinosis, and respiratory disorder
• rachitic chest deformity, and/or vitamin B_6 dependent seizures and failure to thrive

[80] The ENB-002-08 study was conducted for 24 weeks (6 months), and then the ENB-003-08 study was started as the extension study. The study is planned to continue until asfotase alfa is approved and released to the market.

Table 12. RGI-C scores at respective time points (Study ENB-002-08/ENB-003-08)

		Week 4 (n = 10)	Week 12 (n = 10)	Week 24 (n = 10)	Week 36 (n = 9)	Week 48 (n = 9)	Week 72 (n = 9)	Week 144 (n = 8)
Median		0.17	1.17	2.00	2.00	2.67	2.00	2.50
(min, max)		(0.00, 1.00)	(-1.00, 2.00)	(0.00, 2.33)	(1.00, 3.00)	(1.33, 3.00)	(1.67, 3.00)	(1.67, 3.00)
Score category[a]	-3 to <-2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-2 to <-1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-1 to <0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	0 to <1	9 (90.0)	3 (30.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	1 to <2	1 (10.0)	5 (50.0)	2 (20.0)	2 (22.2)	1 (11.1)	2 (22.2)	2 (25.0)
	2 to <3	0 (0.0)	1 (10.0)	7 (70.0)	6 (66.7)	5 (55.6)	6 (66.7)	3 (37.5)
	3	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	3 (33.3)	1 (11.1)	3 (37.5)

a) Score category, number of subjects (proportion [%])

The median change in RSS score (minimum, maximum) from baseline to Weeks 12, 24, 36, 48, 72, and 144 were -1.00 (-4.0, 3.5), -4.00 (-8.0, 0.0), -6.00 (-9.5, 0.0), -6.50 (-10.0, 0.0), -7.00 (-9.5, 0.0), and -6.25 (-9.5, 0.0), respectively.

As to respiratory function, 5 subjects required respiratory assistance at baseline. At Week 24, 3 subjects required a ventilator[81]; 5 subjects required supplemental oxygen only; and 2 subjects were independent of respiratory assistance. Of 9 subjects who participated in Study ENB-003-08 after completing Study ENB-002-08, 8 subjects needed to have some type of respiratory assistance during the study period. Seven of these 8 subjects became independent of respiratory assistance during the administration period and remained independent of it until the final time point (Weeks 144-216). The other 1 subject was weaned from invasive mechanical ventilation and needed no respiratory assistance other than supplemental oxygen until the final time point.

As to growth, the median Z score (minimum, maximum) for height at baseline and at Weeks 24, 48, 72, and 120 was -3.72 (-9.2, -0.7), -3.62 (-8.2, -1.8), -2.85 (-9.2, -1.2), -2.00 (-9.5, -1.2), and -2.44 (-8.6, -0.9), respectively; and that for body weight was -3.84 (-5.4, -0.5), -4.35 (-6.4, -1.5), -3.30 (-6.3, -1.7), -2.96 (-5.3, -0.9), and -1.93 (-4.2, 0.5), respectively.

In analyses of biochemical markers, the plasma PPi concentration (mean ± standard deviation) at baseline and at Weeks 24, 48, and 96 was 5.59 ± 2.26, 2.01 ± 1.39, 2.57 ± 0.96, and 2.78 ± 1.44 μM, respectively, with the change from baseline being -3.30 ± 3.48 at Week 24, -2.96 ± 3.04 at Week 48, and -2.61 ± 1.61 μM at Week 96. The plasma PLP concentration at baseline and at Weeks 24, 48, and 96 was 380.0 ± 256.7, 207.8 ± 460.5, 97.5 ± 131.1, and 93.2 ± 84.9 ng/mL, respectively, with the change from baseline being -181.8 ± 571.1 at Week 24, -282.0 ± 313.6 at Week 48, and -356.1 ± 270.3 ng/mL at Week 96.

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[81] Of the 3 subjects requiring a ventilator at Week 24, 2 subjects had been on a ventilator since baseline.

In the safety analysis,[82] 534 adverse events were reported in 11 of 11 subjects (100%), and 96 adverse drug reactions were reported in 10 of 11 subjects (90.9%). The adverse events reported in ≥3 subjects and adverse drug reactions are shown in Table 13.

Table 13. Adverse events reported in ≥3 subjects and adverse drug reactions
(Study ENB-002-08/003-08)

	Adverse event	Adverse drug reaction
Overall events	11 (100.0)	10 (90.9)
Pyrexia	7 (63.6)	3 (27.3)
Craniosynostosis	6 (54.5)	1 (9.1)
Upper respiratory tract infection	6 (54.5)	0 (0.0)
Otitis media	6 (54.5)	0 (0.0)
Vomiting	5 (45.5)	3 (27.3)
Pneumonia	5 (45.5)	0 (0.0)
Constipation	5 (45.5)	0 (0.0)
Irritability	4 (36.4)	3 (27.3)
Haemoglobin decreased	4 (36.4)	0 (0.0)
Nasopharyngitis	4 (36.4)	0 (0.0)
Rash	4 (36.4)	0 (0.0)
Injection site haematoma	3 (27.3)	2 (18.2)
Urine calcium/creatinine ratio increased	3 (27.3)	1 (9.1)
Headache	3 (27.3)	1 (9.1)
Tooth loss	3 (27.3)	0 (0.0)
Sinusitis	3 (27.3)	0 (0.0)
Respiratory distress	3 (27.3)	0 (0.0)
Wheezing	3 (27.3)	0 (0.0)
Diarrhoea	3 (27.3)	0 (0.0)
Tracheitis	3 (27.3)	0 (0.0)
Fall	3 (27.3)	0 (0.0)
Drug dependence	3 (27.3)	0 (0.0)

Number of subjects with adverse events or adverse drug reactions (incidence [%]); MedDRA/J ver. 13.0

Death was reported in 1 subject,[83] who developed severe septic shock on Day 214. After discontinuation of study treatment, the subject died of pneumonia and septic shock, which were not assessed as adverse drug reactions. Sixty-four serious adverse events were reported in 10 of 11 subjects (90.9%), including the following events reported in ≥2 subjects: 6 episodes of craniosynostosis in 5 subjects; 5 episodes of pneumonia in 3 subjects; 4 episodes of respiratory distress in 2 subjects; and 2 episodes each of convulsion, intracranial pressure increased, and hypoxia in 2 subjects each. Six adverse events leading to study discontinuation were reported in 3 subjects (piloerection, irritability, pyrexia, chills; septic shock; pneumonia).

A total of 51 episodes of injection site reactions (all the episodes occurred after subcutaneous administration) were reported in 6 of 11 subjects (54.5%). They comprised 18 episodes of injection site erythema in 2 subjects, 5 episodes of injection site haematoma in 2 subjects, 5 episodes of injection site swelling in 2 subjects, 3 episodes of injection site nodule in 2 subjects, 3 episodes of injection site warmth in 2 subjects, 3 episodes of injection site cellulitis in 1 subject, 2 episodes each of injection site induration, injection site pruritus, injection site rash, and injection site reaction (PT) in 1 subject each, and 1 episode each of injection site calcification, injection site discolouration, injection site

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[82] The total administration period in the safety analysis population was 26.69 person-years. The number of subjects by administration period was as follows: <3 months, 1; 6 to <9 months, 1; 12 to <24 months, 2; 24 to <36 months, 2; 36 to <48 months, 4; and 48 to 60 months, 1.

[83] The subject had multiple infections including severe pneumonia and tracheitis throughout the study period.

inflammation, injection site pain, injection site papule, and lipohypertrophy in 1 subject each. These adverse events were either mild or moderate in severity. The following 24 episodes of injection-related reactions were reported in 7 of 11 subjects (63.6%) (17 episodes in 5 subjects after intravenous administration[84] and 7 episodes in 3 subjects after subcutaneous administration[85]): 3 episodes each of vomiting, irritability, and pyrexia in 3 subjects each, 2 episodes each of headache and erythema in 1 subject each, and 1 episode each of nausea, tachycardia, chills, myalgia, agitation, cough, tachypnea, hyperhidrosis, increased tendency to bruise, piloerection, and flushed face in 1 subject each. They were all mild or moderate in severity. As ectopic calcification, a total of 2 episodes of nephrocalcinosis were reported in 2 of 11 subjects (18.2%), which were not assessed as adverse drug reactions.

The serum calcium level (individual levels[86] or mean ± standard deviation) was 2.57 ± 0.25 mmol/L at baseline and 2.44 to 2.61 ± 0.09 mmol/L from Week 2 to Week 216. The urine calcium/creatinine ratio (mean[87] or mean ± standard deviation) was 1.15 mmol/mmol at baseline and 0.64 ± 0.47 to 2.20 ± 1.03 mmol/mmol from Week 2 to Week 216.

Six of 10 subjects tested positive for anti-asfotase alfa antibodies with the maximum antibody titer being 2048. Four of these 6 subjects tested positive for neutralizing antibodies.

4.(iii).A.(4) Clinical studies in foreign patients with infantile and childhood hypophosphatasia (5.3.5.1.1, ENB-006-09/ENB-008-10 [ongoing since September 2009; data cut-off, ▮▮▮▮ 20▮▮])
The efficacy and safety of asfotase alfa were investigated in an open-label study[88] enrolling foreign patients with infantile and childhood hypophosphatasia who were aged 5 to 12 years[89] (target sample size of 13). Sixteen patients[90] whose data on the natural history met the inclusion criteria were included in the historical control group. Study ENB-006-09 (24-week study) was followed by Study ENB-008-10, an ongoing extension study.[91]

Asfotase alfa was administered subcutaneously at 2 mg/kg or 3 mg/kg 3 times weekly in Study ENB-006-09, and at the starting dose of 1 mg/kg 3 times weekly for 3 to 9 months and then either at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly in Study ENB-008-10.

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[84] Three episodes of vomiting in 3 subjects, 2 episodes of irritability in 2 subjects, 2 episodes of pyrexia in 2 subjects, and 1 episode each of erythema, nausea, tachycardia, chills, agitation, cough, tachypnoea, hyperhidrosis, piloerection, and flushed face in 1 subject each

[85] Two episodes of headache in 1 subject, and 1 episode each of irritability, pyrexia, erythema, myalgia, and increased tendency to bruise in 1 subject each

[86] 1 subject

[87] 2 subjects

[88] Key inclusion criteria: patients aged 5 to 12 years with Tanner stage of ≤2 (prepubertal) and with the following conditions
  • a serum ALP level below the age-adjusted normal range
  • a plasma PLP level at least twice the upper limit of normal
  • a serum 25(OH) vitamin D level of ≥20 ng/mL
  • rachitic findings related to hypophosphatasia on skeletal radiographs

[89] Subjects were randomized to either the asfotase alfa 6 mg/kg/week group or 9 mg/kg/week group.

[90] ▮▮

[91] Study ENB-006-09 was conducted for 24 weeks (6 months), and then Study ENB-008-10 was started as the extension study. The study is planned to continue until asfotase alfa is approved and released to the market.

In Study ENB-006-09, all of the 13 randomized and treated subjects (6 in the 6 mg/kg/week group [infantile, 3; childhood, 3] and 7 in the 9 mg/kg/week group [infantile, 2; childhood, 5]) were included in the safety analysis population and the FAS. The FAS was defined as the efficacy analysis population. One subject was withdrawn from the study due to corrective surgery for scoliosis. Twelve subjects who completed Study ENB-006-09 were enrolled in Study ENB-008-10.

The median RGI-C score from baseline to Week 24 (minimum. maximum), the primary endpoint, was 2.00 (0.0, 2.3) in the asfotase alfa-treated population (pooled data of the 6 mg/kg/week and 9 mg/kg/week groups) and 0.00 (-1.3, 2.0) in the historical control population, demonstrating a statistically significant difference between these 2 populations ($P = 0.0007$, a two-sided significance level of 5%, LOCF, Wilcoxon signed-rank test). The corresponding value for each of the 2 asfotase alfa groups was 2.00 (1.0, 2.3) in the 6 mg/kg/week group and 2.00 (0.0, 2.3) in the 9 mg/kg/week group. The time course of RGI-C scores from baseline at respective time points for each dose group are shown in Table 14.

Table 14. RGI-C scores at respective time points for each dose group
(Study ENB-006-09/ENB-008-10, FAS)

		Week 24			Week 48			Week 96		
		Historical control (n = 16)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 7)	Historical control (n = 16)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 7)	Historical control (n = 16)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 7)
Median (min, max)		0.00 (-1.3, 2.0)	2.00 (1.0, 2.3)	2.00 (0.0, 2.3)	0.33 (-1.3, 2.0)	1.67 (1.3, 2.0)	1.67 (0.0, 2.3)	0.00 (-1.0, 2.0)	1.67 (1.0, 2.0)	2.00 (0.0, 2.3)
Score category[a]	-3 to <-2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-2 to <-1	1 (6.3)	0 (0.0)	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	-1 to <0	5 (31.3)	0 (0.0)	0 (0.0)	3 (18.8)	0 (0.0)	0 (0.0)	7 (46.7)	0 (0.0)	0 (0.0)
	0 to <1	5 (31.3)	0 (0.0)	1 (14.3)	7 (43.8)	0 (0.0)	1 (14.3)	4 (26.7)	0 (0.0)	1 (14.3)
	1 to <2	4 (25.0)	2 (33.3)	1 (14.3)	4 (25.0)	4 (66.7)	3 (42.9)	3 (20.0)	3 (50.0)	0 (0.0)
	2 to <3	1 (6.3)	4 (66.7)	5 (71.4)	1 (6.3)	2 (33.3)	3 (42.9)	1 (6.7)	3 (50.0)	6 (85.7)
	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

a) Score category, number of subjects (proportion [%]); wk, week

Concerning the main secondary endpoints, the median change in RSS score (minimum, maximum) from baseline to Weeks 24, 48, and 96 was 0.00 (-1.0, 1.5), -0.50 (-1.0, 1.5), and 0.00 (-1.5, 1.5), respectively, in the history control population and -1.50 (-3.5, -0.5), -1.25 (-3.0, 0.0), and -2.00 (-3.5, 0.5), respectively, in the asfotase alfa-treated population. The corresponding values for each of the 2 asfotase alfa groups were -1.25 (-3.5, -0.5), -1.25 (-2.5, 0.0), and -1.50 (-3.0, 0.5), respectively, in the 6 mg/kg/week group and -1.75 (-2.5, -1.0), -1.75 (-3.0, -0.5), and -2.00 (-3.5, -1.5), respectively, in the 9 mg/kg/week group.

Mineralization was evaluated by iliac crest bone biopsy. The osteoid thickness (mean ± standard deviation), the index for the quantity of non-mineralized bone matrix, at baseline and Week 24 was 11.7 ± 3.37 μm and 7.98 ± 2.72 μm, respectively, in the 6 mg/kg/week group and 14.8 ± 2.58 μm and 10.6 ± 6.02 μm, respectively, in the 9 mg/kg/week group, with the change from baseline being -3.75 ± 2.90 μm in the 6 mg/kg/week group and -3.97 ± 5.64 μm in the 9 mg/kg/week group. The ratio of osteoid to bone (mean ± standard deviation) at baseline and Week 24 was 9.98% ± 4.01% and 6.57% ± 2.79%, respectively, in the 6 mg/kg/week group and 17.0% ± 8.04% and 9.62% ± 9.28%, respectively, in the 9

mg/kg/week group, with the change from baseline to Week 24 being -3.42% ± 3.36% in the 6 mg/kg/week group and -5.03% ± 10.6% in the 9 mg/kg/week group.

The results of evaluation of secondary endpoints for growth and biochemical markers (Z-scores for height and body weight, and plasma PPi and PLP concentrations) are shown in Table 15.

Table 15. Results for secondary endpoints
(Z-scores for height and body weight, plasma PPi and PLP concentrations)

	Baseline		Week 24		Week 48		Week 96	
	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 7)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 6)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 6)	6 mg/kg/wk (n = 6)	9 mg/kg/wk (n = 6)
Z-score for height	-1.30 (-6.6, -0.6)	-1.26 (-3.8, 0.0)	-1.28 (-6.9, -0.5)	-1.04 (-3.8, -0.1)	-1.22 (-6.9, -0.2)	-0.91 (-3.5, -0.3)	-1.19 (-6.5, 0.0)	-0.72 (-3.2, -0.1)
Z-score for body weight	-1.52 (-8.2, -1.0)	-0.96 (-3.5, 2.3)	-1.31 (-7.7, 0.0)	-0.43 (-2.8, 1.8)	-1.20 (-7.8, 0.2)	-0.44 (-3.5, 1.9)	-1.07 (-6.6, 0.6)	-0.36 (-2.0, 2.1)
Plasma PPi concentration (μM)	4.57 ± 0.67	5.39 ± 1.08	2.78 ± 0.46	3.23 ± 0.64	2.48 ± 0.37	2.93 ± 0.57	4.08 ± 0.79	4.63 ± 0.67
	–	–	-1.80 ± 0.51	-1.97 ± 0.94	-2.09 ± 0.72	-2.28 ± 0.74	-0.50 ± 1.03	-0.57 ± 0.80
Plasma PLP concentration (ng/mL)	217.5 ± 163.8	211.8 ± 98.4	44.0 ± 20.1	50.7 ± 27.5	27.6 ± 17.4	47.6 ± 32.1[a]	46.3 ± 13.6	71.6 ± 29.2
	–	–	-173.5 ± 154.6	-155.6 ± 91.6	-189.8 ± 168.8	-183.1 ± 94.7[a]	-171.1 ± 155.7	-134.7 ± 90.3

Median (minimum, maximum), Z-scores for height and body weight
Mean ± standard deviation, plasma PPi and PLP concentrations (upper row, measured value; lower row, change from baseline)
–, not applicable
a) n = 5

The results of safety analysis [92] showed 246 adverse events in 6 of 6 subjects (100%) in the 6 mg/kg/week group and 210 adverse events in 7 of 7 subjects (100%) in the 9 mg/kg/week group; and 118 adverse drug reactions in 6 of 6 subjects (100%) in the 6 mg/kg/week group and 111 adverse drug reactions in 6 of 7 subjects (85.7%) in the 9 mg/kg/week group. The adverse events reported in ≥2 subjects in either group and adverse drug reactions are shown in Table 16.

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[92] The total administration period in the safety analysis population was 35.41 person-years. The administration period was <3 months for 1 subject and ≥36 months for 12 subjects.

Table 16. Adverse events reported in ≥2 subjects in either group and adverse drug reactions
(Study ENB-006-09/ENB-008-10)

	Adverse event		Adverse drug reaction	
	6 mg/kg/week	9 mg/kg/week	6 mg/kg/week	9 mg/kg/week
Overall adverse events	6 (100.0)	7 (100.0)	6 (100.0)	6 (85.7)
Injection site erythema	5 (83.3)	7 (100.0)	5 (83.3)	6 (85.7)
Upper respiratory tract infection	5 (83.3)	4 (57.1)	0 (0.0)	0 (0.0)
Vomiting	5 (83.3)	1 (14.3)	0 (0.0)	0 (0.0)
Injection site macule	4 (66.7)	5 (71.4)	4 (66.7)	5 (71.4)
Injection site pruritus	4 (66.7)	3 (42.9)	4 (66.7)	3 (42.9)
Procedural pain	4 (66.7)	3 (42.9)	0 (0.0)	0 (0.0)
Pain in extremity	4 (66.7)	1 (14.3)	0 (0.0)	0 (0.0)
Headache	3 (50.0)	4 (57.1)	0 (0.0)	0 (0.0)
Injection site pain	3 (50.0)	3 (42.9)	3 (50.0)	3 (42.9)
Injection site discolouration	3 (50.0)	1 (14.3)	3 (50.0)	1 (14.3)
Arthralgia	3 (50.0)	1 (14.3)	0 (0.0)	0 (0.0)
Myalgia	3 (50.0)	1 (14.3)	0 (0.0)	0 (0.0)
Fall	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lipohypertrophy	2 (33.3)	3 (42.9)	2 (33.3)	3 (42.9)
Seasonal allergy	2 (33.3)	2 (28.6)	0 (0.0)	0 (0.0)
Gastroenteritis	2 (33.3)	2 (28.6)	0 (0.0)	0 (0.0)
Cough	2 (33.3)	2 (28.6)	0 (0.0)	0 (0.0)
Oropharyngeal pain	2 (33.3)	1 (14.3)	0 (0.0)	0 (0.0)
Injection site atrophy	2 (33.3)	1 (14.3)	2 (33.3)	1 (14.3)
Conjunctival deposit	2 (33.3)	2 (28.6)	2 (33.3)	2 (28.6)
Limb injury	2 (33.3)	1 (14.3)	0 (0.0)	0 (0.0)
Arthropod bite	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhoea	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Nasopharyngitis	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Injection site swelling	1 (16.7)	2 (28.6)	1 (16.7)	2 (28.6)
Fatigue	1 (16.7)	2 (28.6)	0 (0.0)	0 (0.0)
Gait disturbance	0 (0.0)	3 (42.9)	0 (0.0)	0 (0.0)
Epistaxis	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)
Gastrooesophageal reflux disease	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)
Excoriation	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)
Muscular weakness	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)
Rhinorrhoea	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)

Number of subjects (incidence [%]); MedDRA/J ver. 13.0

There were no deaths, serious adverse events, or adverse events leading to discontinuation.

The following 114 episodes of injection site reactions were reported in 6 of 6 subjects (100%) in the 6 mg/kg/week group: 31 episodes of injection site erythema in 5 subjects, 29 episodes of injection site macule in 4 subjects, 20 episodes of injection site pruritus in 4 subjects, 11 episodes of injection site pain in 3 subjects, 8 episodes of injection site discolouration in 3 subjects, 6 episodes of lipohypertrophy in 2 subjects, 3 episodes of injection site atrophy in 2 subjects, 3 episodes of injection site swelling in 1 subject, and 1 episode each of injection site induration, injection site nodule, and injection site papule in 1 subject each. The following 106 episodes of injection site reactions were reported in 6 of 7 subjects (85.7%) in the 9 mg/kg/week group: 39 episodes of injection site erythema in 6 subjects, 34 episodes of injection site macule in 5 subjects, 7 episodes each of injection site pain and lipohypertrophy in 3 subjects each, 3 episodes of injection site pruritus in 3 subjects, 9 episodes of injection site swelling in 2 subjects, 4 episodes of injection site discolouration in 1 subject, and 3 episodes of injection site atrophy in 1 subject. All of these injection site reactions were either mild or moderate in severity. The injection-related reactions reported in each group were as follows: 5 episodes (nausea, oedema peripheral, injection site swelling, injection site erythema, injection site pain) in 1 of 6 subjects (16.7%) in the 6 mg/kg/week group and 5 episodes (injection site erythema; injection site pain, injection site pruritus, injection site erythema; injection site erythema) in 3 of 7 subjects (42.9%) in the 9 mg/kg/week group.

All these injection-related reactions were assessed as adverse drug reactions except for injection site erythema in 1 subject in the 9 mg/kg/week group. As ectopic calcification, 2 episodes of conjunctival deposit were reported in 2 subjects in both the 6 mg/kg/week and 9 mg/kg/week groups. All of these episodes were assessed as adverse drug reactions, but were mild in severity.

The serum calcium level (mean ± standard deviation) at baseline and at Weeks 6 to 168 was 2.51 ± 0.10 mmol/L and 2.43 ± 0.11 to 2.53 ± 0.13 mmol/L, respectively, in the 6 mg/kg/week group, and 2.48 ± 0.09 mmol/L and 2.42 ± 0.09 to 2.55 ± 0.11 mmol/L, respectively, in the 9 mg/kg/week group. The urine calcium/creatinine ratio (mean ± standard deviation) at baseline and at Weeks 1 to 168 was 0.67 ± 0.40 mmol/mmol and 0.28 ± 0.23 to 0.92 ± 0.38 mmol/mmol, respectively, in the 6 mg/kg/week group, and 0.59 ± 0.21 mmol/mmol and 0.19 ± 0.16 to 0.79 ± 0.37 mmol/mmol, respectively, in the 9 mg/kg/week group.

All of the 12 evaluable subjects (6 in each group) tested positive for anti-asfotase alfa antibodies with the maximum antibody titer being 2048. Two subjects in the 9 mg/kg/week group tested positive for neutralizing antibodies.

4.(iii).A.(5) Clinical studies in foreign patients with infantile, childhood, and adult hypophosphatasia (5.3.5.1.2, ENB-009-10 [ongoing since June 2010; data cut-off, ███ 20█])
The efficacy and safety of asfotase alfa were investigated in a non-treatment controlled, randomized, open-label study enrolling foreign patients with infantile, childhood, and adult hypophosphatasia who were aged 13 to 65 years[93] (target sample size of 19). The study is currently underway.[94]

Subjects received asfotase alfa subcutaneously at 0.3 or 0.5 mg/kg once daily for 24 weeks. Those who were not treated with asfotase alfa were included in the control group. Then, all subjects received asfotase alfa subcutaneously at 0.5 mg/kg once daily from Week 24 to Week 48, and at 1 mg/kg 6 times weekly after Week 48.[95] It was permitted to adjust the dose in response to the change in body weight every 3 months up to 80 mg/day.

All of the 19 subjects treated (6 in the control group [infantile, 1; childhood, 5]; 7 in the 0.3 mg/kg/day group [infantile, 1; childhood, 3; adult, 2; unknown time of onset, 1]; and 6 in the 0.5 mg/kg/day group [infantile, 2; childhood, 4]) were included in the safety analysis population and the FAS. The FAS was defined as the efficacy analysis population.

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[93] Key inclusion criteria: patients aged 13 to 65 years with the following conditions, including the 4 patients who were enrolled in Study ENB-001-08 and completed the study
  • a serum ALP level below the age-adjusted normal range
  • a plasma PLP level at least twice the upper limit of normal
  • an evidence of osteopenia or osteomalacia on skeletal radiographs
  • osteomalacia on iliac crest bone biopsy, characterized by a mineralization lag time (MLT) z-score of ≥2
[94] The study is planned to continue until asfotase alfa is approved and released to the market only if PPi is not sufficiently inhibited.
[95] In accordance with the revised protocol, asfotase alfa was subcutaneously administered to some subjects at 0.5 mg/kg once daily for several weeks after Week 48.

Changes in plasma PPi and PLP concentrations from baseline to Week 24 were defined as co-primary endpoints. The median change in plasma PLP concentration from baseline to Week 24 (minimum, maximum) was 11.00 (-374.0, 346.0) ng/mL in the control population and -254.5 (-1467.0, -17.2) ng/mL in the asfotase alfa-treated population (pooled data of the 0.3 mg/kg/day group and the 0.5 mg/kg/day group), showing a statistically significant difference between the 2 populations ($P = 0.0285$, a two-sided significance level of 5%, LOCF, Wilcoxon signed-rank test). Meanwhile, the median change in plasma PPi concentration from baseline to Week 24 (minimum, maximum) was -0.18 (-6.84, 1.07) μM in the control population and -2.19 (-4.40, 0.32) μM in the asfotase alfa-treated population, showing no statistically significant difference between the 2 populations ($P = 0.0715$, a two-sided significance level of 5%, LOCF, Wilcoxon signed-rank test). These results of analysis of the co-primary endpoints did not demonstrate the superiority of treatment with asfotase alfa over non-treatment in terms of efficacy. The change in plasma PPi concentration [mean ± standard deviation]) from baseline to Weeks 24, 48, and 96 was -2.03 ± 1.44, -1.49 ± 1.37, and -2.06 ± 3.25 μM, respectively, in the 0.3 mg/kg/day group, and -2.19 ± 1.33, -1.28 ± 1.54, and -2.69 ± 2.08 μM, respectively, in the 0.5 mg/kg/day group. The change in plasma PLP concentration (mean ± standard deviation) from baseline to Weeks 24, 48, and 96 was -255.0 ± 196.2, -235.3 ± 191.6, and -301.3 ± 252.6 ng/mL, respectively, in the 0.3 mg/kg/day group, and -564.3 ± 624.0, -593.4 ± 605.2, and -584.6 ± 654.9 ng/mL, respectively, in the 0.5 mg/kg/day group.

Main secondary endpoints were evaluated by iliac crest bone biopsy.[96] The osteoid thickness (mean ± standard deviation) in the control group, 0.3 mg/kg/day group, and 0.5 mg/kg/day group was 12.7 ± 1.75, 9.10 ± 4.41, and 9.03 ± 5.11 μm, respectively, at baseline and 11.5 ± 6.87, 9.53 ± 5.70, and 7.52 ± 3.44 μm, respectively, at Week 24 (control group) or Week 48 (asfotase alfa groups), with the change from baseline being -1.13 ± 6.09, -0.01 ± 3.63, and -1.52 ± 2.36 μm, respectively. The ratio of osteoid to bone (mean ± standard deviation) in the control group, 0.3 mg/kg/day group, and 0.5 mg/kg/day group was 11.6% ± 4.53%, 6.53% ± 4.59%, and 6.55% ± 3.48%, respectively, at baseline and 11.8% ± 7.82%, 8.39% ± 4.81%, and 3.70% ± 2.18%, respectively, at Week 24 (control group) or Week 48 (asfotase alfa groups), with the change from baseline being 0.20% ± 4.77%, 1.21% ± 3.25%, and -2.85% ± 2.38%, respectively. The bone mineral content measured using dual-energy X-ray absorptiometry (DEXA) at baseline and Week 24 was 1775 ± 604 and 2012 ± 682 g, respectively, in the control group, 2391 ± 782 and 2369 ± 779 g, respectively, in the 0.3 mg/kg/day group, and 2309 ± 537 and 2323 ± 485 g, respectively, in the 0.5 mg/kg/day group, with the change from baseline being 237 ± 414 (the control group), -21.9 ± 35.4 (the 0.3 mg/kg/day group), and 41.5 ± 20.0 g (the 0.5 mg/kg/day group).

The 6-minute walking distance (mean ± standard deviation) at baseline and Week 24 was 217.8 ± 218.9 m and 327.8 ± 220.9 m, respectively, in the control group, 380.3 ± 158.3 m and 445.0 ± 131.3 m, respectively, in the 0.3 mg/kg/day group, and 444.5 ± 118.3 m and 488.0 ± 116.6 m, respectively, in the

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[96] Subjects in the control group received asfotase alfa at 0.5 mg/kg/day after completion of the Week 24 visit. Therefore, these endpoints in the control group were evaluated using data obtained at Week 24.

0.5 mg/kg/day group, with the change from baseline being 13.5 ± 69.8 (the control group), 64.7 ± 73.0 (the 0.3 mg/kg/day group), and 43.5 ± 43.2 m (the 0.5 mg/kg/day group).

The results of safety analysis[97] were as follows: adverse events included 46 episodes in 6 of 6 subjects (100%) in the control group, 234 episodes in 7 of 7 subjects (100%) in the 0.3 mg/kg/day group, and 80 episodes in 6 of 6 subjects (100%) in the 0.5 mg/kg/day group; and adverse drug reactions included 1 episode in 1 of 6 subjects (16.7%) in the control group, 130 episodes in 4 of 7 subjects (57.1%) in the 0.3 mg/kg/day group, and 12 episodes in 2 of 6 subjects (33.3%) in the 0.5 mg/kg/day group. The adverse events reported in ≥2 subjects in any group and adverse drug reactions are shown in Table 17.

Table 17. Adverse events reported in ≥2 subjects in any group and adverse drug reactions (Study ENB-009-10)

	Adverse event			Adverse drug reaction		
	Control	0.3 mg/kg/day	0.5 mg/kg/day	Control	0.3 mg/kg/day	0.5 mg/kg/day
Overall events	6 (100.0)	7 (100.0)	6 (100.0)	1 (16.7)	4 (57.1)	2 (33.3)
Injection site pain	0 (0.0)	4 (57.1)	0 (0.0)	0 (0.0)	4 (57.1)	0 (0.0)
Injection site erythema	0 (0.0)	3 (42.9)	2 (33.3)	0 (0.0)	3 (42.9)	2 (33.3)
Injection site haematoma	0 (0.0)	3 (42.9)	1 (16.7)	0 (0.0)	3 (42.9)	1 (16.7)
Injection site pruritus	0 (0.0)	3 (42.9)	0 (0.0)	0 (0.0)	3 (42.9)	0 (0.0)
Pain in extremity	1 (16.7)	2 (28.6)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Joint swelling	0 (0.0)	2 (28.6)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Back pain	1 (16.7)	2 (28.6)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Arthralgia	1 (16.7)	2 (28.6)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Oropharyngeal pain	0 (0.0)	2 (28.6)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	1 (16.7)	2 (28.6)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Injection site swelling	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)
Injection site discolouration	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)
Cough	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dysmenorrhoea	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Neck pain	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sinusitis	0 (0.0)	2 (28.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Bone pain	0 (0.0)	1 (14.3)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	0 (0.0)	0 (0.0)	3 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Nasopharyngitis	2 (33.3)	0 (0.0)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)

Number of subjects (incidence [%]); MedDRA/J ver. 13.0

There were no deaths or adverse events leading to study discontinuation. The serious adverse events reported were as follows: 4 episodes (staphylococcal infection, humerus fracture, femur fracture, gastroenteritis) in 2 subjects in the control group and 6 episodes (convulsion after dosing at 0.3 g/kg/day; and staphylococcal infection, device dislocation; pain in extremity, muscular weakness; and adrenal adenoma after dosing at 0.5 mg/kg/day) in 4 subjects in the asfotase alfa-treated population.[98] None of these serious adverse events were assessed as adverse drug reactions.

The injection site reactions reported were as follows: 1 episode (injection site reaction [PT]) in 1 of 6 subjects (16.7%) in the control group; 130 episodes (61 episodes of injection site erythema in 3 subjects, 17 episodes of injection site pain in 4 subjects, 16 episodes of injection site pruritus in 3 subjects, 14 episodes of injection site haematoma in 3 subjects, 10 episodes of injection site swelling in 2 subjects, 9 episodes of injection site discolouration in 2 subjects, and 3 episodes of injection site induration in 1

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[97] Safety data up to Week 24
[98] Including safety data after Week 24

subject) in 4 of 7 subjects (57.1%) in the 0.3 mg/kg/day group; and 12 episodes (5 episodes of injection site erythema in 2 subjects, 2 episodes of injection site haematoma in 1 subject, and 1 episode of injection site papule in 1 subject) in 2 of 6 subjects (33.3%) in the 0.5 mg/kg/day group. In addition, 2 episodes of lipohypertrophy were reported in 1 subject in each group. No injection-related reactions were reported. As ectopic calcification, the following episodes were reported: 2 episodes of deposit eye in 2 subjects in the control group, 2 episodes of deposit eye in 2 subjects in the 0.3 mg/kg/day group, and 1 episode each of deposit eye and conjunctival deposit in 2 subjects in the 0.5 mg/kg/day group. All of these episodes were assessed as adverse drug reactions, but were mild in severity.

The serum calcium level (mean ± standard deviation) at baseline in the control group, 0.3 mg/kg/day group, and 0.5 mg/kg/day group was 2.52 ± 0.04, 2.45 ± 0.07, and 2.43 ± 0.09 mmol/L, respectively. The serum calcium level ranged from 2.47 ± 0.04 to 2.54 ± 0.05 mmol/L between Weeks 6 and 24 in the control group, from 2.37 ± 0.10 to 2.47 ± 0.07 mmol/L between Weeks 3 and 24 in the 0.3 mg/kg/day group, and from 2.26 ± 0.25 to 2.42 ± 0.08 mmol/L between Weeks 3 and 24 in the 0.5 mg/kg/day group. The urine calcium/creatinine ratio (mean ± standard deviation) at baseline in the control group, 0.3 mg/kg/day group, and 0.5 mg/kg/day group was 0.47 ± 0.47, 0.19 ± 0.21, and 0.35 ± 0.08 mmol/mmol, respectively. The urine calcium/creatinine ratio ranged from 0.18 ± 0.14 to 0.42 ± 0.49 mmol/mmol between Weeks 6 and 24 in the control group, from 0.19 ± 0.18 to 0.32 ± 0.38 mmol/mmol between Weeks 3 and 24 in the 0.3 mg/kg/day group, and from 0.16 ± 0.09 to 0.24 ± 0.10 mmol/mmol between Weeks 3 and 24 in the 0.5 mg/kg/day group.

Five of the 6 subjects in the control group, 5 of 7 subjects in the 0.3 g/kg/day group, and 5 of 6 subjects in the 0.5 mg/kg/day group tested positive for anti-asfotase alfa antibodies, with the maximum antibody titer being 256. Two subjects in the 0.5 mg/kg/day group tested positive for neutralizing antibodies.

4.(iii).B   Outline of the review by PMDA
4.(iii).B.(1) Clinical positioning
The applicant explained as follows:

Hypophosphatasia is a genetic disorder caused by loss-of-function mutations in the gene encoding tissue non-specific alkaline phosphatase (TNSALP).[1] Major clinical signs include progressive multiple organ dysfunction such as deformity and destruction of bones, pain, marked muscle weakness, respiratory failure, convulsive seizures, renal impairment, and dental abnormalities. Among non-adult patients, the major cause of death is respiratory failure,[2] for which the mortality in this patient population is 50% to 100%[3] in the most severe cases. Fraser proposed the following classification of common forms of hypophosphatasia: infantile, manifesting immediately after birth or during the first 6 months of life; childhood, gradually manifesting after the first 6 months of life; and adult, manifesting in adulthood.[99] Subsequently Whyte et al. proposed another classification comprising 7 types including perinatal, manifesting in the fetal period.[100] In the submitted clinical studies, hypophosphatasia was classified by

[99] Fraser D. *Am J Med*. 1957;22:730-46
[100] Whyte MP. *Pediatric Bone: Biology and Disease*. Glorieux FH, et al eds. London, Academic Press, 2012;771-94

age of onset mainly into infantile (including perinatal), childhood, and adult types. It is considered that infantile hypophosphatasia is generally the most severe type, and that symptoms differ among different disease types and vary even within the same disease type, while different disease types may share some symptoms. Given that the primary etiology shared by all disease types is impaired mineralization due to increases in the concentrations of TNSALP substrates, all disease types can be discussed collectively as one disease.

In Japan, no drug product has been approved for the indication of hypophosphatasia, and the following symptomatic treatment options are currently in use: mechanical ventilation by endotracheal intubation; calcium-restricted diets or diuretic administration to remove calcium; administration of vitamin B_6 preparations for convulsive seizures; and surgical intervention for craniosynostosis.[6] Administration of parathyroid hormone (PTH) preparations in adult patients on a trial basis has been suggested to relieve bone pain and to improve fracture healing and ambulation, all to a limited extent,[101] but no results have been reported concerning improvement of bone mineralization.

Asfotase alfa is a preparation of recombinant human TNSALP-Fc-deca-aspartate fusion protein, which is believed to induce normal skeletal development by the following mechanism: asfotase alfa, an enzyme preparation, replaces deficient TNSALP, which hydrolyses PPi, a TNSALP substrate that inhibits bone mineralization, and the resulting inorganic phosphate (Pi) binds to calcium, thereby promoting formation of HA crystals and bone mineralization. Since no effective drug products are approved for the treatment of hypophosphatasia, asfotase alfa is expected to be an important treatment option for the disease.

PMDA agrees that hypophosphatasia is a serious disease, and since no drugs have been approved with an indication for this disease in Japan, PMDA also acknowledges that asfotase alfa, which replaces deficient TNSALP, is a new treatment option to improve the symptoms of hypophosphatasia and that it is significant to make asfotase alfa available to healthcare providers in clinical settings.

4.(iii).B.(2) Efficacy
Given that hypophosphatasia is a rare and serious disease, PMDA included foreign clinical studies in the evaluation of the efficacy of asfotase alfa. In the global clinical study (Study ENB-010-10), the number of Japanese participants was as small as 5. Therefore, the entire subject population was included in the efficacy analysis, and the efficacy in Japanese subjects was evaluated individually.

The applicant explained as follows:
In infantile and childhood hypophosphatasia, impaired mineralization of bone matrix manifests as the following rachitic signs: curved legs; swelling of wrist, knee, and ankle joints resulting from

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[101] Camacho PM, et al. *Endocr Pract*. 2008;14(2):204-8; Doshi KB, et al., *Clin Cases Miner Bone Metab*. 2009;6(3):266-9; Gagnon C, et al., *J Clin Endocrinol Metab*. 2010;95(3):1007-12; Laroche M. *Calcif Tissue Int*. 2012;90(3):250; Schalin-Jäntti C, et al. *J Clin Endocrinol Metab*. 2010;95(12):5174-9; Silva I, et al. *Acta Reumatol Port*. 2012;37(1):92-6; Whyte MP, et al. *J Clin Endocrinol Metab*. 2007;92(4):1203-8

metaphyseal enlargement; and bead-like enlargement of costochondral junctions. Therefore, an objective evaluation of the effects of asfotase alfa on skeletal abnormalities responsible for developmental and functional impairments was made on X-ray images obtained from pediatric subjects with open growth plates, before and after the start of asfotase alfa administration, using the RGI-C score[60] and the RSS score.[61] At each time point, the results demonstrated an improvement in terms of rachitic signs, shown as an increased RGI-C score and a decreased change in RSS score from baseline (Table 18). Also the plasma PPi and PLP concentrations, which indicate any decrease in the activity of alkaline phosphatase (ALP), are believed to increase in the presence of loss-of-function mutations in the gene encoding TNSALP. Elevated PPi levels are known to inhibit bone mineralization, thereby causing skeletal deformities and deposition of excessive non-mineralized matrix (osteoid), and elevated PLP levels are associated with B_6-dependent convulsive seizures.[14] The levels of these biochemical markers measured in clinical studies revealed that the plasma PPi and PLP concentrations were reduced at Week 24, and remained low thereafter (Table 18).

Table 18. Changes from baseline in main efficacy endpoints

Age range		≤5 years		5-12 years	13-66 years
Study		ENB-002-08/ ENB-003-08	ENB-010-10	ENB-006-09/ ENB-008-10	ENB-009-10
RGI-C score	Week 24	2.00 (0.00, 2.33) (n = 10)	1.83 (-0.33, 3.00) (n = 26)	2.00 (1.0, 2.3) (n = 12)	–
	Week 48	2.67 (1.33, 3.00) (n = 9)	2.00 (1.67, 3.00) (n = 15)	1.67 (1.3, 2.3) (n = 12)	–
	Week 96	2.00 (2.00, 3.00) (n = 9)	2.67 (1.67, 3.00) (n = 9)	2.00 (1.0, 2.3) (n = 12)	–
RSS score	Week 24	-4.00 (-8.0, 0.0) (n = 9)	-1.00 (-7.5, 4.0) (n = 25)	-1.50 (-3.5, -0.5) (n = 12)	–
	Week 48	-6.50 (-10.0, 0.0) (n = 9)	-4.00 (-9.5, -0.5) (n = 12)	-1.25 (-3.0, 0.0) (n = 12)	–
	Week 96	-6.50 (-10.0, 0.0) (n = 9)	-1.50 (-8.0, 1.5) (n = 11)	-2.00 (-3.5, 0.5) (n = 12)	–
Plasma PPi (μM)	Week 24	-3.30 ± 3.48 (n = 7)	-3.11 ± 2.69 (n = 26)	-1.88 ± 0.73 (n = 12)	-1.95 ± 1.24 (n = 19)
	Week 48	-2.96 ± 3.04 (n = 7)	-2.21 ± 2.41 (n = 15)	-2.19 ± 0.71 (n = 12)	-1.42 ± 1.40 (n = 19)
	Week 96	-2.61 ± 1.61 (n = 7)	-0.26 ± 7.00 (n = 11)	-0.53 ± 0.88 (n = 12)	-2.35 ± 2.68 (n = 13)
Plasma PLP (ng/mL)	Week 24	-181.8 ± 571.1 (n = 8)	-2185.8 ± 4091.2 (n = 17)	-164.5 ± 121.5 (n = 12)	-341.9 ± 410.0 (n = 19)
	Week 48	-282.0 ± 313.6 (n = 7)	-1644.8 ± 2787.5 (n = 11)	-186.8 ± 133.6 (n = 11)	-342.8 ± 387.8 (n = 17)
	Week 96	-356.1 ± 270.3 (n = 6)	-3916.6 ± 6039.6 (n = 9)	-152.9 ± 122.8 (n = 12)	-432.1 ± 481.9 (n = 13)

RGI-C score and RSS score as median (minimum, maximum); PPi and PLP as mean ± standard deviation; –, not calculated

In Study ENB-006-09/ENB-008-10 enrolling patients aged 5 to 12 years with hypophosphatasia, bone mineralization was evaluated as follows: the quantity of non-mineralized bone matrix (osteoid thickness and volume) was measured by iliac crest bone biopsy, and bone mineral content was measured using DEXA. The results showed that the quantity of non-mineralized bone matrix (osteoid thickness and volume) had a tendency to decrease with a corresponding increase in bone mineral content. Other findings included improved growth, shown by increases in the Z-scores for height and body weight, and increased 6-minute walk distances [see "4.(iii).A.(4) Foreign clinical studies in patients with infantile and childhood hypophosphatasia"]. Efficacy was also evaluated in a pooled analysis of subjects with

infantile (n = 44) and childhood (n = 8) hypophosphatasia enrolled in Studies ENB-010-10, ENB-002-08, and ENB-006-09. The results showed that in both populations, the median RGI-C score from baseline to Week 24 was 2.00, and continued to increase thereafter. The changes in RSS score also showed a tendency to decrease in the severity of rickets. The Z-scores for height and weight also increased, and similar clinical effects were achieved in the patients with either type of disease.

It is known that hypophosphatasia is life-threatening in the most severe cases, and often results in a fatal outcome due to respiratory failure secondary to pulmonary hypoplasia and rachitic mechanical thoracic damage.[102] Therefore, survival and ventilator-free survival were compared between subjects with infantile hypophosphatasia in Studies ENB-010-10 and ENB-002-08/ENB-003-08 (n = 37, the asfotase alfa-treated population) and those enrolled in Study ENB-011-10 in which natural clinical course of hypophosphatasia[103] was accumulated (n = 48, the control population).[104] Survival tended to improve in the asfotase alfa-treated population versus with the control population, as shown by the following survival rates (number at risk, number of deaths, number censored) estimated using the Kaplan-Meier method: 95% (34, 2, 1) versus 42% (20, 28, 0), respectively, at Week 48 from baseline; 89% (27, 4, 6) versus 31% (14, 33, 1), respectively, at Week 96 from baseline; 89% (19, 4, 14) versus 27% (11, 35, 2), respectively, at Week 192 from baseline; and 89% (3, 4, 30) versus 27% (7, 35, 6), respectively, at Week 384 from baseline.[105] Then the survival in the asfotase alfa-treated population (n = 37) was compared with those calculated from the data of control patients (25 of 48 subjects) who survived at least 38 weeks after birth (the median weeks of age, when enrolled, in the asfotase alfa-treated population) using the Kaplan-Meier method. Patients in the control population were stratified by time of diagnosis of hypophosphatasia because the later their diagnosis was made the longer they survived. Again the results showed that survival tended to improve in the asfotase alfa-treated population compared with the control population. Ventilator-free survival tended to improve in the asfotase alfa-treated population versus to the control population, as shown by the following ventilator-free survival rates estimated using the

[102] Caswell AM, et al. *Crit Rev Clin Lab Sci*. 1991;28(3):175-232; Whyte MP. *Pediatric Bone: Biology and Diseases*. Glorieux FH, et al eds. London, Academic Press, 2012;771-94

[103] Key inclusion criteria: patients with onset of hypophosphatasia prior to 6 months of age having a medical record including hypophosphatasia as indicated by 1 or more of the following (i) to (iii) and perinatal and infantile hypophosphatasia as indicated by 1 or more of the following (iv) to (vi)

     (i) ALPL gene mutation(s)

     (ii) a serum ALP level below the age-adjusted normal range and either plasma PLP or urinary phosphoethanolamine (PEA) above the upper limit of normal

     (iii) a serum ALP level below the age-adjusted normal range and hypophosphatasia-related radiographic abnormalities on X-ray

     (iv) respiratory compromise (up to and including respiratory failure) requiring institution of respiratory support measure(s), requiring medication(s) for management of symptom(s), and/or associated with other respiratory complications

     (v) vitamin B_6-responsive seizures

     (vi) rachitic chest deformity

[104] The age of onset of hypophosphatasia (mean ± standard deviation) of the asfotase alfa-treated population and the control population was 1.42 ± 1.72 months of age (n = 26) and 1.12 ± 1.67 (n = 47) months of age, respectively, in the aggregated data. In both populations, those who had a history of rachitic chest deformity or respiratory disorder accounted for ≥73% and those who had vitamin B_6-responsive seizures accounted for <36%. In the asfotase alfa-treated population, ≥50% had complications such as failure to thrive and/or poor body weight gain, developmental delay, muscular weakness, and nephrocalcinosis, and ≥32% had a history of fracture or bone pain. In the aggregated data, it was found that 23% to 76% of the control population had such complications.

[105] Baseline was defined for the asfotase alfa-treated population and the control population as the date of starting treatment with asfotase alfa and the date of birth, respectively.

Kaplan-Meier method: 96% (23, 1, 1) versus 31% (15, 33, 0), respectively, at Week 48 from baseline; 83% (18, 4, 3) versus 25% (11, 36, 1), respectively, at Week 96 from baseline; 83% (15, 4, 6) versus 25% (10, 36, 2), respectively, at Week 192 from baseline; and 83% (3, 4, 18) versus 25% (6, 36, 6), respectively, at Week 384 from baseline.[105]

The asfotase alfa-treated (infantile, 5; childhood, 8) and control (infantile, 7; childhood, 9) populations in Study ENB-006-09/ENB-008-10 were also compared in terms of the severity of rickets (RGI-C score). The median RGI-C scores at Weeks 24, 48, 96 from baseline for infantile hypophosphatasia were 2.00, 1.67, and 1.00, respectively, in the asfotase alfa-treated population and 0.00, 0.00, and -0.67, respectively, in the control population; and those for childhood hypophosphatasia were 2.00, 2.00, and 2.00 respectively, in the asfotase alfa-treated population and 0.00, 0.33, and 0.33, respectively, in the control population.

The above results of clinical studies and the data for the natural clinical course of hypophosphatasia obtained from Study ENB-011-10 can be interpreted as demonstrating the efficacy of asfotase alfa in the treatment of hypophosphatasia.

PMDA asked the applicant to justify the use of the RGI-C score, the primary endpoint in Studies ENB-010-10, ENB-002-08, and ENB-006-09, in evaluating the efficacy of asfotase alfa.

The applicant responded as follows:
In infantile and childhood hypophosphatasia, impaired mineralization of bone matrix manifests as the following rachitic signs: curved legs; swelling of wrist, knee, and ankle joints resulting from metaphyseal enlargement; and bead-like enlargement of costochondral junctions. In the absence of indices to assess skeletal manifestations of hypophosphatasia over time, the RGI-C score is considered to allow assessment of the essential pathology of hypophosphatasia, including skeletal changes such as metaphyseal fraying, osteopenia, and metaphyseal enlargement, in patients of different ages including infants and children. Therefore, in the main clinical studies, the RGI-C score in patients with open growth plates and aged <18 years was used as the primary endpoint. Skeletal manifestations of hypophosphatasia were assessed by independent reviewers as follows: 3 trained radiologists (blinded to imaging time points after baseline and other patient information) rated the RGI-C score by comparing X-ray images taken at baseline and during study treatment. The inter-observer agreement was evaluated using a 2-factor analysis of variance model, and the intraclass correlation coefficient was 0.65 for Studies ENB-002-08 and ENB 010-10 (pooled data, assessed by the same review team) and 0.57 for Study ENB-006-09 (assessed by another review team). These results can be interpreted as suggesting adequate reproducibility of the RGI-C score, and that the RGI-C score thus allows evaluation of overall changes in skeletal manifestations including those of rickets.

PMDA asked the applicant to explain the differences in intrinsic and extrinsic ethnic factors and in characteristics between Japanese and foreign patients, and also to explain the efficacy in Japanese

patients.

The applicant responded as follows:
There are no major differences in diagnostic approach[106] or in treatment approach to hypophosphatasia between Japan and other countries. The symptoms of hypophosphatasia vary in severity among individual patients, but are similar between in and out of Japan.

The factors underlying the use of asfotase alfa were compared between Japan and other countries. No major pharmacokinetic differences were found between Japanese and foreign subjects [see "4.(ii).B.(1) Pharmacokinetics in Japanese and foreign patients"]. Among patient characteristics in Study ENB-010-10, baseline age (mean ± standard deviation) was higher in Japanese subjects (143.1 ± 123.7 weeks, n = 5) than that in the entire subject population (118.2 ± 113.5 weeks, n = 28). The median Z-scores for baseline height and weight were -2.92 (n = 27) and -2.41 (n = 28), respectively, in the entire subject population and -5.41 (n = 5) and -5.87 (n = 5), respectively, in Japanese subjects. The median RSS score was 4.50 (n = 27) in the entire subject population and 7.00 (n = 5) in Japanese subjects. Plasma PPi concentration (mean ± standard deviation) was 6.82 ± 2.15 μM (n = 28) in the entire subject population and 6.38 ± 0.65 μM (n = 5) in Japanese subjects. Plasma PLP concentration (mean ± standard deviation) was 5083 ± 7130 ng/mL (n = 24) in the entire subject population and 9488 ± 7023 ng/mL (n = 4) in Japanese subjects. Japanese subjects, albeit limited in number, had thus higher baseline severity indices than the entire subject population, except for plasma PPi concentration.

Regarding efficacy in Japanese subjects, the RGI-C score at Week 24 from baseline, the primary endpoint, ranged from 0.67 to 3.00 as shown in Table 9 and suggested a tendency for the severity of rickets to decrease although no significant difference was observed due to the limited number of subjects. Also the changes in plasma PPi and PLP concentrations showed a tendency to decrease from baseline (-1.90 to -5.46 μM and -2575 to -5737 ng/mL, respectively) at all the time points (from Week 6 to Week 48 of treatment).

PMDA considers as follows:
There are no established indices to evaluate the therapeutic time course of hypophosphatasia, and comparability is limited in the data for the natural clinical course of hypophosphatasia obtained from patients enrolled in Study ENB-011-10. Therefore, it is difficult to evaluate the efficacy of asfotase alfa rigorously. However, in clinical studies, rachitic symptoms and other findings based on diagnostic X-ray imaging tended to decrease in severity; the plasma concentrations of the 2 biochemical markers, PPi and PLP, also decreased; and survival tended to be higher in subjects treated with asfotase alfa than in patients whose data for the natural clinical course of hypophosphatasia are available in Study ENB-011-10. These findings can be interpreted as reasonably demonstrating the efficacy of asfotase alfa in the treatment of hypophosphatasia. Tendencies in rachitic symptoms and biochemical markers observed in

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[106] Study Report for Fiscal Year 2011 of Research on Optimal Personalized Treatment of Hypophosphatasia, Health and Labour Sciences Research Grants, Research Project on Treatment of Intractable Diseases; Whyte, MP, et al. *N Engl J Med*. 2012;366:904-13

foreign subjects were similarly observed in Japanese subjects, albeit limited in number, and therefore asfotase alfa is expected to be effective also in Japanese patients. Because the number of subjects analyzed was small, it is necessary to continue to collect information on the efficacy of asfotase alfa through post-marketing surveillance. These discussions will be finalized after taking into account the comments made in the Expert Discussion.

4.(iii).B.(3) Safety
The applicant provided the following explanation.

Table 19 shows the incidence of adverse events for respective disease types in a pooled analysis of Studies ENB-010-10, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, and ENB-009-10. Commonly reported adverse events (≥25 episodes per 100 person-years) were as follows (the number in the parentheses is the number of episodes per 100 person-years): injection site erythema (153.8), upper respiratory tract infection (84.6), pyrexia (57.7), injection site discolouration (52.9), headache (36.5), injection site induration (32.7), tooth loss (31.7), and vomiting (31.7) for infantile hypophosphatasia; and injection site erythema (186.0), injection site macule (86.9), injection site reaction (PT) (55.6), upper respiratory tract infection (53.9), injection site discolouration (46.9), injection site pain (45.2), injection site pruritus (43.5), erythema (43.5), pain in extremity (36.5), contusion (26.1), and injection site swelling (26.1) for childhood hypophosphatasia. Injection site bruising occurred in both of 2 subjects with adult hypophosphatasia. The adverse event reported at a high incidence among the entire subject population was injection site reaction.[75] In 32 of 71 subjects (45.1%), 183 non-fatal serious adverse events (108.1 episodes per 100 person-years) were reported. Most of these episodes were hypophosphatasia-related symptoms or complications such as craniosynostosis, pneumonia, and respiratory disorder. They included the following 11 episodes of adverse drug reactions reported in 4 subjects: chronic hepatitis; craniosynostosis and conductive deafness; chills and pyrexia (Japanese subjects); and hypoaesthesia oral, pain in extremity, and chills (3 events), and headache. In the Japanese subject population, the following 11 serious adverse events were reported in 4 of 5 subjects: 3 episodes of respiratory tract inflammation in 1 subject, and 1 episode each of pyrexia, chills, hydrocephalus, bronchitis, influenza, mechanical ventilation complication, performance of nuclear magnetic resonance imaging (MRI), and surgery of the respiratory tract in 1 subject each. Of these, chills and pyrexia reported in 1 subject were assessed as adverse drug reactions.

Table 19. Incidence of adverse events (pooled analysis for respective disease types)

	Infantile	Childhood	Adult	Unknown time of onset	Overall
Number of subjects evaluated (exposure person-years)	48 (104.0 PY)	20 (57.5 PY)	2 (5.0 PY)	1 (2.8 PY)	71 (169.3 PY)
All adverse events	48 (100.0) 1776.7	20 (100.0) 1307.0	2 (100.0) 515.3	1 (100.0) 2901.7	71 (100.0) 1597.9
All adverse drug reactions	37 (77.1) 458.6	20 (100.0) 703.9	2 (100.0) 336.9	1 (100.0) 253.9	60 (84.5) 535.0
Nonfatal serious adverse events	27 (56.3) 165.4	5 (25.5) 19.1	0 (0.0) 0	0 (0.0) 0	32 (45.1) 108.1
Adverse events leading to study discontinuation	3 (6.3) 8.7	0 (0.0) 0	0 (0.0) 0	0 (0.0) 0	3 (4.2) 5.3

PY, person-years; Upper row, number of subjects with adverse events (incidence [%]); Lower row, number of episodes per 100 person-years[107]

An investigator-initiated clinical study of asfotase alfa in patients with hypophosphatasia is currently underway in Japan. At present, safety data up to ███ █, 20█ have been obtained from 7 subjects receiving asfotase alfa subcutaneously at 2 mg/kg 3 times weekly (age at the start of treatment, █ days old to 17 years old; administration period, approximately 1 week to 1 year and 9 months). In this study, 55 adverse events were reported in 7 of 7 subjects (100.0%). The adverse events reported in ≥2 subjects were 13 episodes of injection site erythema in 4 subjects, 8 episodes of upper respiratory tract infection in 3 subjects, 6 episodes of injection site induration in 2 subjects, 2 episodes of enterocolitis in 2 subjects, and 2 episodes of exanthema subitum in 2 subjects. Injection site reactions were observed in many subjects at an early stage of treatment; however, none of these reactions worsened over the course of further treatment. Four serious adverse events (hypocalcaemia, convulsion, mixed deafness, and acute upper respiratory tract inflammation) were reported in 1 subject, and the hypocalcaemia and convulsion were assessed as adverse drug reactions. Within the currently available safety data, there were no adverse events leading to dose modification or study discontinuation.

In the study, Japanese subjects were initially required to receive asfotase alfa in an inpatient or outpatient setting if they had participated in Study ENB-010-10. This dosing requirement was later changed to self-administration as in other clinical studies of asfotase alfa in consideration of the potential burden on subjects. No safety issues or problems related to injection technique have been reported from these subjects up to this time. More than half of the subjects participating in the Japanese investigator-initiated study are receiving asfotase alfa via self-administration. No problems have been reported in these subjects.

Taking into account the incidence of adverse events reported in subjects of clinical studies including Japanese subjects, PMDA considers that the safety of treatment with asfotase alfa is acceptable if an appropriate precautionary statement is provided based on the review of the following events, 1) to 4).

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[107] Annual incidence of adverse events in each subject was calculated by dividing the number of adverse events in the subject by the exposure period (weeks) and then by multiplying the quotient by 52. Then, the mean of the annual incidences in all subjects was calculated.

4.(iii).B.(3).1) Injection site reactions

The applicant provided the following explanation:

In a pooled analysis of Studies ENB-010-10, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, and ENB-009-10, there were 815 episodes (481.3 episodes per 100 person-years) of injection site reactions in 52 of 71 subjects (73.2%). The injection site reactions that occurred in ≥5 subjects are summarized in Table 20.

Table 20. Injection site reactions that occurred in ≥5 subjects (pooled analysis)

	Number of subjects (Incidence [%])	Number of episodes (Number of episodes/100 person-years)
Injection site erythema	37 (52.1)	270 (159.4)
Injection site discolouration	17 (23.9)	82 (48.4)
Injection site pain	16 (22.5)	51 (30.1)
Injection site pruritus	14 (19.7)	46 (27.2)
Injection site macule	11 (15.5)	75 (44.3)
Injection site swelling	11 (15.5)	34 (20.1)
Injection site bruising	9 (12.7)	25 (14.8)
Injection site hypertrophy	9 (12.7)	29 (17.1)
Injection site induration	9 (12.7)	39 (23.0)
Injection site reaction	9 (12.7)	40 (23.6)
Injection site atrophy	8 (11.3)	20 (11.8)

MedDRA version 16.1

Many of the injection site reactions observed were mild and resolved spontaneously, and none of them were assessed as serious adverse events. There were 6 episodes of injection site reactions leading to dose reduction (injection site erythema and injection site macule; and injection site discolouration, injection site atrophy, injection site pruritus, and injection site reaction [PT]) reported in 2 subjects with childhood hypophosphatasia, 1 of whom discontinued the study treatment after consent withdrawal.

The incidence of injection site reactions was as follows: 413 episodes (397.1 episodes per 100 person-years) in 29 of 48 subjects with infantile hypophosphatasia; 380 episodes (660.5 episodes per 100 person-years) in 20 of 20 subjects with childhood hypophosphatasia; and 15 episodes (297.3 episodes per 100 person-years) in 2 of 2 subjects with adult hypophosphatasia.

Twelve episodes (7.1 episodes per 100 person-years) of lipohypertrophy at the injection site were reported in 5 of 71 subjects (7.0%). They were 10 episodes (9.6 episodes per 100 person-years) in 4 of 48 subjects with infantile hypophosphatasia and 2 episodes (3.5 episodes per 100 person-years) in 1 of 20 subjects with childhood hypophosphatasia. Most episodes were found in the upper extremity or abdomen and were either mild or moderate in severity. None of the episodes were serious.

As stated above, no serious adverse events have been reported in patients treated with asfotase alfa. However, taking into account the relatively high incidence of injection site reactions, the following precautionary statement will be provided in the package insert and other relevant materials: injection site should be rotated each time. Information related to these adverse events will continue to be collected through the post-marketing surveillance.

PMDA considers as follows:

Although no serious adverse events have been reported, injection site reactions occurred most frequently in clinical studies. Therefore, a precautionary statement regarding injection site reactions should be appropriately provided, and further information needs to be collected via post-marketing surveillance.

4.(iii).B.(3).2) Injection-related reactions

The applicant provided the following explanations:

In a pooled analysis of Studies ENB-010-10, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, and ENB-009-10, there were 51 episodes of injection-related reactions (30.1 episodes per 100 person-years) in 16 of 71 subjects (22.5%). The main injection-related reactions reported in ≥3 subjects were as follows: 4 episodes of injection site erythema (2.4 episodes per 100 person-years) in 4 of 71 subjects (5.6%), 4 episodes of pyrexia (2.4 episodes per 100 person-years) in 4 of 71 subjects (5.6%), 5 episodes of chills (3.0 episodes per 100 person-years) in 3 of 71 subjects (4.2%), 3 episodes of irritability (1.8 episodes per 100 person-years) in 3 of 71 subjects (4.2%), and 3 episodes of vomiting (1.8 episodes per 100 person-years) in 3 of 71 subjects (4.2%). Most of these episodes were mild in severity and were associated with intravenous administration.[84] As serious adverse events, 8 episodes of injection-related reactions (chills and pyrexia [Japanese subject]; and hypoaesthesia oral, pain in extremity, chills [3 episodes], and headache), which were associated with subcutaneous administration, were reported in 2 subjects with infantile and childhood hypophosphatasia. As adverse events leading to study discontinuation, 4 episodes of injection-related reactions (piloerection, irritability, pyrexia, and chills), which were associated with intravenous administration, were reported in 1 subject with infantile hypophosphatasia. This subject was withdrawn from the study. As adverse events leading to modification of the administration method, 6 episodes of intravenous injection-related reactions (pyrexia, stomatitis, tachycardia, tachypnea, and vomiting; and vomiting) were reported in 2 subjects with infantile hypophosphatasia. The intravenous infusion rate was modified for 1 of these 2 subjects, and intravenous administration was discontinued for the other subject.

Forty episodes (38.5 episodes per 100 person-years) of injection-related reactions were reported in 13 of 48 subjects with infantile hypophosphatasia, and 11 episodes (19.1 episodes per 100 person-years) of injection-related reactions in 3 of 20 subjects with childhood hypophosphatasia.

There were no anaphylactic reactions to asfotase alfa when it was administered subcutaneously in clinical studies.

PMDA understands the applicant's explanation that most of the injection-related reactions observed in clinical studies were associated with intravenous administration. However, given that Japanese subjects receiving subcutaneous injections experienced serious adverse events and that injection-related reactions were also reported after subcutaneous administration, it will be necessary to provide adequate precautions regarding injection-related reactions and to continue to collect relevant information in the post-marketing surveillance.

4.(iii).B.(3).3) fluctuations in serum calcium levels

The applicant provided the following explanation:

In a pooled analysis of Studies ENB-010-10, ENB-002-08/ENB-003-08, ENB-006-09/ENB-008-10, and ENB-009-10, the serum calcium levels ranged from 1.92 to 4.03 mmol/L at baseline, from 1.82 to 2.80 mmol/L at Week 24, and from 2.12 to 3.67 mmol/L at the last visit. Although they varied widely at baseline, their intra-individual variability remained small throughout the treatment period, showing no tendency to increase or decrease excessively. Asfotase alfa induced an increase in bone calcification as observed radiographically, and the change was accompanied by a tendency toward normalization of serum calcium levels throughout the treatment period. As events considered to be related to abnormal calcium levels, 25 adverse events related to ectopic calcification were reported in 22 of 71 subjects (31.0%). Of these, the adverse events reported in ≥2 of 71 subjects were deposit eye in 8 subjects, conjunctival deposit and nephrocalcinosis in 6 subjects each, and corneal deposits in 2 subjects. All the 25 episodes of ectopic calcification were either mild or moderate in severity, and 1 episode of nephrocalcinosis and many episodes of calcification of the eye were assessed as adverse drug reactions. Visual disturbance was not found in subjects who experienced the calcification of the eye. There were no clear relationships between serum calcium levels and the onset of ectopic calcification. It is known that calcification of the eye can be caused by abnormal calcium homeostasis associated with hypophosphatasia. [108] Nephrocalcinosis has also been considered one of the complications of hypophosphatasia.[100] In Study ENB-011-10 enrolling patients as the source of the natural clinical course of hypophosphatasia, 16 of 31 subjects (51.6%) had experienced nephrocalcinosis during a period from the perinatal to 5 years of age. In addition, 20 of 59 subjects (33.9%) in clinical studies of asfotase alfa were reported to have a medical history of nephrocalcinosis specific to hypophosphatasia, and 8 of 11 subjects (72.7%) in Study ENB-002-08 had a history of nephrocalcinosis. These data suggest that the onset of ectopic calcification is not specifically related to treatment with asfotase alfa.

PMDA asked the applicant to discuss whether asfotase alfa may cause fluctuations in serum calcium levels which result in serious symptoms, and to explain whether it is necessary to provide precautions regarding fluctuations in serum calcium levels.

The applicant responded as follows:

In clinical studies, 3 subjects experienced hypercalcaemia; 2 subjects experienced hypocalcaemia; and 1 subject experienced both of them. All these events were mild or moderate in severity. The hypocalcaemia reported in 2 subjects was assessed as an adverse drug reaction, and one of these subjects experienced the event during intravenous administration. None of these fluctuations in serum calcium levels caused adverse events that were serious or that led to study discontinuation or dose modification.[109]

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[108] Roxburgh ST. *Trans Ophthalmol Soc UK*. 1983;103(Pt 5):513-6

[109] The clinical course of 1 subject who developed hypocalcaemia was as follows: the subject developed it on Day 50 and severe septic shock on Day 214, which led to study discontinuation; the subject died of pneumonia and complications of septic shock. The clinical course of 1 subject who developed both hypercalcaemia and hypocalcaemia was as follows: the subject developed hypercalcaemia on Day 2 and hypocalcaemia on Day 27, and died of cardiorespiratory arrest on Day 436. None of these events were assessed as adverse drug reactions.

In the ongoing Japanese investigator-initiated clinical study in patients with hypophosphatasia, serious hypocalcaemia was reported after dosing in 1 subject at the age of 23 days. The subject, who started to receive asfotase alfa on the day of birth subcutaneously at 2 mg/kg 3 times weekly, had extremely low PTH at birth and had hypercalcaemia (up to 12 mg/dL) from 2 to 4 days after birth. A decrease in serum calcium (the reported minimum level, 4.7 mg/dL) was observed after administration of asfotase alfa, and convulsive seizures probably caused by the decreased serum calcium occurred at 28 days after birth. The subject started to take supplemental calcium at 7 days after birth, and the serum calcium level became normal at 43 days after birth (supplemental calcium was discontinued then) and remained normal thereafter. Hypocalcaemia and convulsion in the subject were assessed as adverse drug reactions, but did not lead to dose modification.

As stated above, a large fluctuation in serum calcium levels is expected at an early stage of treatment with asfotase alfa due to its rapid effect on the normalization of bone metabolism. Therefore, it is desirable to monitor serum calcium level during treatment with asfotase alfa. A caution statement regarding fluctuations in serum calcium levels, including its monitoring, will be provided.

PMDA considers as follow:
Given that fluctuations in serum calcium levels were observed in subjects treated with asfotase alfa in clinical studies, it will be necessary to provide appropriate precautions in the package insert and to ensure that serum calcium levels are monitored during treatment in consideration of safety.

4.(iii).B.(3).4) Effects of antibody production
The applicant explained as follows:
Of 69 subjects with post-baseline antibody data in clinical studies, 56 subjects (81.2%) (infantile, 36; childhood, 18; and adult, 2) tested positive for anti-asfotase alfa antibodies at some post-baseline time point. Among these 56 subjects, 25 (infantile, 20; childhood, 4; and adult, 1) also showed the presence of neutralizing antibodies at some post-baseline time point. The antibody titers ranged from 0 to 2048, and the median time to the first anti-asfotase alfa antibody positive test result was 37 days (ranged from 14 to 1072 days).

In an analysis of the relationships between antibody production and the severity of rickets reported in clinical studies (based on RGI-C or RSS score), the degree of improvement in rachitic severity was similar between subjects with positive and negative anti-asfotase alfa antibody test results and between subjects with positive and negative neutralizing antibody test results, showing no effects of antibody titers on the efficacy of asfotase alfa.

In a comparison of safety data between subjects who tested positive for anti-asfotase alfa antibodies at least once and those who did not, the incidence of adverse drug reactions was higher in the former population (50 of 56 subjects [89.3%]) than in the latter population (9 of 13 subjects [69.2%]). This

result was considered attributable to a higher incidence of injection site reactions and injection-related reactions in subjects with a positive antibody test result (768 episodes in 44 of 56 subjects [78.6%] and 42 episodes in 12 of 56 subjects [21.4%], respectively) than that in subjects with a negative test result (47 episodes in 8 of 13 subjects [61.5%] and 4 episodes in 2 of 13 subjects [15.4%], respectively), though its cause has not been clarified. Meanwhile, the incidence rate of adverse events was higher in subjects before having the first positive test result (2448.3 episodes per 100 person-years) than after having it (1218.2 episodes per 100 person-years). The incidence rate of "general disorders and administration site conditions" (SOC) was 856.5 episodes per 100 person-years in subjects before having the first positive test result and 387.9 episodes per 100 person-years after having it. The incidence rate of "eye disorders" (SOC) was higher in subjects after having the first positive test result (40.1 episodes per person-years) than before having it (21.2 episodes per 100 person-years), which was likely to be due to the imbalanced incidence of conjunctivitis (1.9 episodes per 100 person-years before the first positive result and 6.0 episodes per 100 person-years after that). The incidence of severe adverse events and serious adverse events was lower in subjects with a positive antibody test result (44 episodes in 18 of 56 subjects [32.1%] and 125 episodes in 23 of 56 subjects [41.1%], respectively) compared to that in subjects with a negative antibody test result (83 episodes in 11 of 13 subjects [84.6%] and 58 episodes in 9 of 13 subjects [69.2%], respectively).

Similarly, safety data were compared between subjects who tested positive for neutralizing antibodies at least once and those who did not. The incidence of adverse drug reactions in the former population (23 of 25 subjects [92.0%]) was higher than in the latter population (36 of 44 subjects [81.8%]). This result was mainly attributable to a higher incidence of injection-related reactions in subjects with a positive antibody test result (32 episodes in 10 of 25 subjects [40.0%]) than that in subjects with a negative test result (14 episodes in 4 of 44 subjects [9.1%]). However, the incidence rate of adverse events was higher in subjects before having the first positive test result (1691.5 episodes per 100 person-years) than after having it (1198.4 episodes per 100 person-years), and the incidence rate of "general disorders and administration site conditions" (SOC) in subjects before having the first positive test result (607.1 episodes per 100 person-years) was approximately 3-fold higher than that in subjects after having it (219.0 episodes per 100 person-years). The incidence rates of erythema, headache, and increased tendency to contusion (PT) included in injection-related reactions in subjects before having the first positive test result (26.4, 33.7, and 4.4 episodes, respectively, per 100 person-years) were higher than that after having it (6.1, 0.0, and 0.0 episodes, respectively, per 100 person-years).

No correlation was detected between anti-asfotase alfa antibody titers and neutralizing antibody titers (percentage inhibition of enzyme activity).

These results of clinical studies suggest that antibody production has no marked effects on the efficacy and safety of asfotase alfa. However, taking into account that asfotase alfa, a protein product, has been reported to produce antibodies and that only limited data have been obtained from clinical studies,

information will continue to be collected in the post-marketing surveillance in terms of effects of antibody production on efficacy and safety.

PMDA considers as follows:

Given that antibody production has been reported after treatment with asfotase alfa and that only a limited number of subjects were investigated in Japanese and foreign clinical studies, information on the impact of anti-drug antibodies should continue to be collected through the post-marketing surveillance.

4.(iii).B.(4) Indications

The applicant explained as follows:

Hypophosphatasia is a disorder caused by a deficiency of TNSALP. The results of clinical studies demonstrated that administration of asfotase alfa that replaces deficient TNSALP improves mineralization abnormalities and associated symptoms in patients with hypophosphatasia. While at least 279 gene mutations have been identified in the TNSALP, no obvious relationships have been found between gene mutations and pathological condition or severity.[110]

Therefore, "hypophosphatasia" is considered appropriate for the indication of asfotase alfa.

PMDA sees no particular problem in that asfotase alfa is indicated for "hypophosphatasia." Nevertheless, a final decision on the appropriateness of this indication will be made after taking account of comments from the Expert Discussion.

4.(iii).B.(5) Dosage and administration

The applicant explained as follows:

Data from non-clinical studies suggested that the concentration of asfotase alfa at which efficacy is expected is 650 to 1000 U/L. Based on data obtained from Study ENB-001-08, the serum asfotase alfa concentration is supposed to be 1000 U/L in patients who receive the drug subcutaneously at 1 mg/kg 7 times weekly or at 2.3 mg/kg 3 times weekly. In Study ENB-002-08/ENB-003-08 in ≤3 year-old patients who developed infantile hypophosphatasia at <6 months of age (n = 11), the starting dose was 1 mg/kg administered subcutaneously 3 times weekly, which was lower dose than that of the estimated effective concentration above. After 1 month of treatment, it was permitted to increase the starting dose according to the results of the efficacy evaluation (maximum dose administered at a 3-timesweekly regimen,[111] 2 mg/kg [n = 7]; 3 mg/kg [n = 2]; and 4 mg/kg [n = 1]), and the majority of the subjects received asfotase alfa subcutaneously at 2 mg/kg 3 times weekly. Although the efficacy of treatment with asfotase alfa at each dose level was not evaluated in this study, the median RGI-C score from baseline to Week 24 (minimum, maximum) was 2.00 (0.00, 2.33), which indicated the effectiveness of asfotase alfa in

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[110] Rockman-Greenberg C. *Pediatric Endocrinokogy Reviews*. 2013;10:380-8
[111] Excluding 1 subject who was withdrawn from the study on Day 1 after receiving an intravenous dose of asfotase alfa

improving the severity of rickets. This beneficial effect was also maintained in the extension study (Table 12).

In Study ENB-006-09 in pediatric patients aged 5 to 12 years who developed hypophosphatasia (infantile, 5; childhood, 8) at ≥6 months of age and in whom growth plates were open at the time of enrollment, asfotase alfa was subcutaneously administered at 2 or 3 mg/kg (2 mg/kg [n = 6], 3 mg/kg [n = 7]) 3 times weekly based on the assumption that bone turnover in children was slower than that in infants. In the subsequent extension study, ENB-008-10, asfotase alfa was subcutaneously administered at 1 mg/kg 3 times weekly for 3 to 9 months. Later, in consideration of the frequent occurrence of pain and plateaued improvement in rickets in some subjects, the dose was increased to 6 mg/kg/week, subcutaneously administered, at the discretion of the investigator, either at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly. As a result, the median RGI-C score from baseline to Week 24 (minimum, maximum) increased to 2.00 (1.0, 2.3) in the 6 mg/kg/week group and 2.00 (0.0, 2.3) in the 9 mg/kg/week group, demonstrating an improvement in the severity of rickets. This beneficial effect was maintained thereafter (Table 14).

In Study ENB-002-08/ENB-003-08, significant improvement was observed in bone mineralization and respiratory function in subjects who received asfotase alfa subcutaneously at 2 mg/kg 3 times weekly. Therefore, in Study ENB-010-10 in ≤5 year-old patients who developed infantile hypophosphatasia at <6 months of age (n = 28), the dose level of asfotase alfa was set at 6 mg/kg/week administered subcutaneously either at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly, which was adjusted according to the results of the efficacy and safety evaluation (starting dose: 2 mg/kg 3 times weekly [n = 27]; 2 mg/kg 7 times weekly [n = 1][112]). The median RGI-C score from baseline to Week 24 (minimum, maximum) was 1.67 (-1.67, 3.00), which indicated the effectiveness of asfotase alfa in improving the severity of rickets. This beneficial effect was maintained thereafter (Table 8). All the Japanese patients (n = 5) enrolled in the study received asfotase alfa subcutaneously at 2 mg/kg 3 times weekly, which was unchanged throughout the study period. Data on biochemical markers obtained from Study ENB-006-09/ENB-008-10 suggested that asfotase alfa was effective when given at a dose lower than 2 mg/kg 3 times weekly. Therefore, in Study ENB-009-10 in pediatric and adult patients with hypophosphatasia (infantile, 4; childhood, 12; adult, 2; unknown time of onset, 1), the dose level of 0.5 mg/kg/day was investigated. In addition, the dose level of 0.3 mg/kg/day, which affected biochemical markers (plasma PPi and PLP concentrations) in Study ENB-001-08, was also investigated (0.3 mg/kg/day [n = 7]; 0.5 mg/kg/day [n = 6]; and non-treatment control [n = 6]). Subjects received subcutaneous doses at 0.5 mg/kg/day from Week 24 to Week 48, and at 1 mg/kg 6 times weekly after Week 48. Because these dosage regimens were different from those in other studies, the results of Study ENB-009-10 were not included in the efficacy data for the pooled analysis. As stated above, the most commonly used dosage regimen in clinical studies was 6 mg/kg/week, which was confirmed to be effective when given at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly.

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[112] In consideration of severity of the disease at enrollment, the starting dosage for this subject was determined to be 2 mg/kg administered subcutaneously 7 times weekly.

Based on the results of a PK/PD analysis performed using an E_{max} model constructed by a PPK analysis, a simulation[113] was performed to examine dose-response relationship in terms of the efficacy and safety in patients with infantile and childhood hypophosphatasia treated with asfotase alfa given at 0.02 to 2 mg/kg 7 times weekly; at 1 mg/kg 6 times weekly; or at 2 mg/kg 3 times weekly. Regarding the respective efficacy endpoint parameters[114] (plasma PPi concentration, plasma PLP concentration, RGI-C score, RSS score, 6-minute walking distance, and Bruininks-Oseretsky test of motor proficiency [BOT-2] score), the effect of asfotase alfa reached a plateau around the dose level of 6 g/kg/week, indicating the effectiveness of the dose. No marked difference in efficacy was detected between subcutaneous administration at 1 mg/kg 6 times weekly and that at 2 mg/kg 3 times weekly. The results of the simulation also suggested that the incidence of adverse events (injection site reactions, injection-related reactions, and ectopic calcification) was not dependent on the level of exposure. According to the results of clinical studies, the incidence of injection site reactions during the first 24 weeks of treatment was 319 episodes (with a rate of 655.2 episodes per 100 person-years) in 15 of 20 subjects (75.0%) at 1 mg/kg 6 times weekly and 582 episodes (with a rate of 482.3 episodes per 100 person-years) in 41 of 50 subjects (82.0%) at 2 mg/kg 3 times weekly. The number of episodes per unit time was larger in subjects treated at 1 mg/kg 6 times weekly, the regimen considered to be commonly employed, than in those treated at 2 mg/kg 3 times weekly. The incidence of serious adverse events during the first 24 weeks of treatment was higher in subjects treated at 2 mg/kg 3 times weekly (19 of 50 subjects [38.0%]) than in those treated at 1 mg/kg 6 times weekly (4 of 20 subjects [20.0%]), probably reflecting not the difference in regimen but the difference in age between the 2 treatment groups (mean age is 3.77 years and 39.96 years, respectively; hypophosphatasia is generally less severe in older patient population).

Hypophosphatasia is classified by age of onset into perinatal, infantile, childhood, and adult types. However, based on the results of clinical studies and in consideration of the potential burden on patients, the proposed dosage and administration was set as follows irrespective of disease types: the total weekly dose of asfotase alfa is 6 mg/kg, which is to be subcutaneously administered either in 3 or 6 divided doses weekly. In routine clinical settings, where the quantity of the drug solution for subcutaneous injection at a time is limited up to 1 mL, asfotase alfa is expected to be administered 3 times weekly to younger patients with low body weight, for whom a lower dose level is sufficient, and 3 or 6 times weekly depending on body weight to older patients who require a higher dose.

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[113] The simulation was performed on the following assumptions: the formulation used was prepared in the batch size of ▮▮ L with total sialic acid content of ▮▮ mol/mol and specific activity of ▮▮ U/mg; patients were tested negative for both anti-drug antibodies and neutralizing antibodies; the age and body weight of patients were the median of those of patients in each onset-time-based hypophosphatasia type.

[114] The simulation was performed to examine plasma PPi concentration, plasma PLP concentration, RGI-C score, and RSS score for infantile and childhood hypophosphatasia, and 6-minute walking distance and BOT-2 score for childhood hypophosphatasia.

On the basis of the results of clinical studies and the simulation for the dose-response relationship, the proposed dosage and administration of asfotase alfa was set at 1 mg/kg administered subcutaneously 6 times weekly or 2 mg/kg administered subcutaneously 3 times weekly.

PMDA asked the applicant to explain the dosage and administration of asfotase alfa for patients with adult hypophosphatasia.

The applicant responded as follows:
There were no patients with adult hypophosphatasia who had been examined in clinical studies except for 2 subjects enrolled in Study ENB-009-10. These subjects received asfotase alfa subcutaneously at 0.3 mg/kg/day once daily until Week 23 or 24; then at a dose increased to 0.5 mg/kg/day until Week 47 or 72; and at 1 mg/kg 6 times weekly thereafter. The plasma PPi concentration at baseline and at Weeks 24 and 96 was 4.06, 2.61, and 0.85 μM, respectively, in one subject and 4.60, 3.58, and 1.91 μM, respectively, in the other subject. The plasma PLP concentration at the corresponding time points was 34.5, 15.7, and 2.89 ng/mL, respectively, in one of the subjects and 577, 141, and 6.71 ng/mL, respectively, in the other subject. As shown in these results, plasma PPi and PLP concentrations in both subjects decreased over time. The main adverse events were 9 episodes of injection site reactions in 1 subject, but these were all mild in severity. In the ongoing Japanese investigator-initiated study in patients with hypophosphatasia, no adverse events have been reported in the adult subject treated with subcutaneous doses of asfotase alfa at 1.18 mg/kg 3 times weekly since January 2015. Hypophosphatasia is a disease characterized by bone mineralization abnormalities caused by a deficiency or loss of TNSALP activity. In patients with adult hypophosphatasia who exhibit no growth of bone due to the disappearance of the growth plate cartilage, treatment with asfotase alfa is unlikely to improve their height, but is expected to improve bone mineralization which may lead to the maintenance of bone homeostasis with a reduced likelihood of fractures and bone pain. Although there is currently limited experience of treatment with asfotase alfa according to the proposed dosage and administration in patients with adult hypophosphatasia, no notable safety problems have been reported, and the treatment is expected to be effective in this patient population.

PMDA considers as follows:
Given that no dose-finding clinical study of asfotase alfa has been conducted and that the doses selected for clinical studies were different from one another, it is difficult to determine the optimal dosage and administration based on the efficacy and safety data obtained from these studies. However, the following applicant's explanation is understandable: the most commonly used dose in the study population including Japanese subjects was 6 mg/kg/week administered at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly, at which dose efficacy has been demonstrated in subjects while no clinically significant problems have been reported with safety, and the results of a simulation also supported the appropriateness of this proposed dosage and administration. Nevertheless, taking into account the high incidence of injection site reactions among patients treated with asfotase alfa, the dose should be reduced according to the patient's condition. The efficacy and safety of asfotase alfa, when administered

according to the proposed dosage and administration, have not been established in patients with adult hypophosphatasia. This information should be provided in the package insert and other relevant materials. Because of the very limited number of Japanese patients included in clinical studies and limited experience of treatment with asfotase alfa in patients with adult hypophosphatasia of any nationality, information on the dosage and administration, efficacy, and safety should continue to be collected through the post-marketing surveillance. These discussions will be finalized after taking into account the comments made in the Expert Discussion.

4.(iii).B.(6) Post-marketing investigations

The applicant explained as follows:

A specified drug use-results survey (survey period, 9 years; follow-up period, from enrollment to the end of the survey period) will be conducted in all patients[115] treated with asfotase alfa. In this survey, the following information will be collected: patient characteristics, immunogenicity, injection site reactions, injection-related reactions, fluctuations in serum calcium levels, and other relevant matters.

PMDA considers as follows:

Taking into account the very limited number of Japanese patients included in clinical studies, information should be collected on the efficacy and safety of asfotase alfa from all patients treated with the drug. A final decision on the appropriateness of this conclusion and the details of the survey such as the method, period, and survey items will be made after taking account of comments expressed in the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based compliance inspection of the data submitted in the new drug application was conducted in accordance with the provisions of the Pharmaceutical Affairs Act. PMDA concluded that there should be no problem in conducting a regulatory review based on the submitted application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

A GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.2.3). Based on the results, PMDA concluded that there should be no problem in conducting a regulatory review based on the submitted application documents.

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[115] In addition to these patients, those who have received a diagnosis of hypophosphatasia but are not on treatment with asfotase alfa will also be surveyed, if permission is obtained from the medical institution.

IV. Overall Evaluation

It is concluded that the submitted data suggest that asfotase alfa (Strensiq) is effective in patients with hypophosphatasia, and its safety is acceptable in view of its observed benefits. Asfotase alfa is thus considered to be a clinically relevant treatment option for hypophosphatasia. PMDA considers that further investigation through post-marketing surveillance is needed for injection-site reactions, injection-related reactions, the effects of fluctuations in serum potassium levels and of antibody production on safety and efficacy, the safety of long-term use, and other issues.

PMDA considers that Strensiq may be approved if it can be concluded based on comments from the Expert Discussion that there are no particular problems.

May 14, 2015

I. Product Submitted for Registration

[Brand name]	Strensiq Subcutaneous Injection 12 mg/0.3 mL,
	Strensiq Subcutaneous Injection 18 mg/0.45 mL,
	Strensiq Subcutaneous Injection 28 mg/0.7 mL,
	Strensiq Subcutaneous Injection 40 mg/1 mL,
	Strensiq Subcutaneous Injection 80 mg/0.8 mL
[Non-proprietary name]	Asfotase Alfa (Genetical Recombination)
[Applicant]	Alexion Pharma G.K.
[Date of application]	October 15, 2014

II. Content of the Review

The outline of the comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) is described in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc. concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc. by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

(1) Efficacy

PMDA considers as follows:

There are no established indices to evaluate the therapeutic time course of hypophosphatasia, and comparison with the data of the natural clinical course of hypophosphatasia obtained from patients enrolled in Study ENB-011-10 is possible only to the limited extent. Therefore, the efficacy of injectable preparations containing Asfotase Alfa (Genetical Recombination; hereinafter, asfotase alfa) as the active ingredient is difficult to evaluate rigorously. However, in clinical studies, rachitic symptoms and other findings evaluated based on radiographic images tended to decrease in severity; the plasma concentrations of the 2 biochemical markers, inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP), also decreased; and in Study ENB-011-10, survival tended to be longer in subjects treated with asfotase alfa than in patients who provided the data for the natural clinical course of hypophosphatasia. These findings can be interpreted as reasonably demonstrating the efficacy of asfotase alfa in the treatment of hypophosphatasia. Similar tendencies in rachitic symptoms and biochemical markers were observed in Japanese subjects, albeit limited in number, and therefore asfotase alfa is expected to be effective also in Japanese patients. Because the number of subjects analyzed was small, it is necessary to continue to collect information on the efficacy of asfotase alfa through post-marketing surveillance.

The above conclusion of PMDA was supported by the expert advisors.

(2) Safety

PMDA considers as follows:

On the basis of the incidence of adverse events in clinical studies including Japanese subjects and the results of analyses of individual events (injection-site reactions, injection-related reactions, effects of fluctuations in serum potassium levels and of antibody production, and other issues), it is concluded that the safety of asfotase alfa in patients with hypophosphatasia is acceptable so long as an appropriate precautionary statement is provided. It is necessary to continue to collect information on injection-site reactions, injection-related reactions, effects of fluctuations in serum potassium levels and of antibody production, and other issues through the post-marketing surveillance.

The above conclusion of PMDA was supported by the expert advisors.

PMDA instructed the applicant to provide an appropriate precautionary statement in the package insert and confirmed that the applicant has followed this instruction accordingly [see "(5) Risk management plan (draft)" for post-marketing investigations].

(3) Indication

The known forms of hypophosphatasia include perinatal, infantile, childhood, adult, tooth-specific (odontohypophosphatasia), and benign perinatal types. [116] In the submitted clinical studies, hypophosphatasia was classified into infantile (including perinatal), childhood, and adult types. PMDA has concluded that there are no particular problems with asfotase alfa being indicated for "hypophosphatasia" based on its mechanism of action, regardless of disease type.

The above conclusion of PMDA was supported by the expert advisors.

(4) Dosage and administration

PMDA considers as follows:

Given that no dose-finding clinical study of asfotase alfa has been conducted and that the doses selected for clinical studies were different from one another, it is difficult to determine the optimal dosage and administration based on the efficacy and safety data obtained from these studies. However, the following applicant's explanation is understandable: the most commonly used dose in the study population including Japanese subjects was 6 mg/kg/week administered at 1 mg/kg 6 times weekly or at 2 mg/kg 3 times weekly, at which dose efficacy has been demonstrated in subjects while no clinically significant problems have been reported with safety, and the results of a simulation also supported the appropriateness of this proposed dosage and administration. Nevertheless, taking into account the high

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[116] Study Report for Fiscal Years 2009 to 2011 of Research on Optimal Personalized Treatment of Hypophosphatasia, Health and Labour Sciences Research Grants, Research Project on Treatment of Intractable Diseases; Fraser D. *Am J Med*. 1957; 22: 730-46; Whyte, MP. *Pediatric Bone: Biology and Disease*. Glorieux FH, et al eds. London, Academic Press, 2012;771-94

incidence of injection site reactions among patients treated with asfotase alfa, the dose should be reduced according to the patient's condition. Judging from the results of Study ENB-009-10 in pediatric and adult patients, the efficacy and safety of asfotase alfa, when administered according to the proposed dosage and administration, have not been established in patients with adult hypophosphatasia. This information should be provided in the package insert and other relevant materials. Because of the very limited number of Japanese patients included in clinical studies and limited experience of treatment with asfotase alfa in patients with adult hypophosphatasia in and out of Japan, information on the dosage and administration, efficacy, and safety should continue to be collected through the post-marketing surveillance.

The above conclusion of PMDA was supported by the expert advisors.

PMDA instructed the applicant to revise the description in the "Dosage and Administration" section as follows:
[Dosage and Administration]
The usual dosage of Asfotase Alfa (Genetical Recombination) is <u>1 mg/kg administered subcutaneously 6 times weekly or 2 mg/kg administered subcutaneously 3 times weekly. The dose may be reduced according to the patient's condition</u>. (The revised parts are underlined.)

The applicant responded that the dosage and administration would be revised as instructed above and the package insert would also be modified accordingly. PMDA accepted the response.

(5) Risk management plan (draft)
On the basis of the review presented in "4.(iii).B.(6) Post-marketing investigations" of the Review Report (1) and comments from the Expert Discussion, PMDA concluded that the risk management plan of asfotase alfa should include the safety and efficacy specifications shown in Table 21 and that the additional pharmacovigilance activities and risk minimization activities shown in Tables 22 and 23, including a specified use-results survey during the re-examination period (all-case surveillance), should be conducted.

Table 21. Safety and efficacy specifications in the risk management plan (draft)

Safety specifications		
Important identified risks	Important potential risks	Important missing information
• Injection site reactions • Injection-related reactions • Hypocalcaemia	• Effects of anti-asfotase alfa antibody production • Ectopic calcification • Craniosynostosis	• Long-term safety
Efficacy specifications		
• Long-term efficacy		

Table 22. Outline of additional pharmacovigilance activities and risk minimization activities in the risk management plan (draft)

Additional pharmacovigilance activities	Additional risk minimization activities
• Early post-marketing phase vigilance • Specified drug use-results survey on long-term treatment (all-case surveillance)	• Early post-marketing phase vigilance • Information provision via materials for healthcare professionals • Information provision via materials for patients

Table 23. Outline of specified use-results survey (draft)

Objectives	To evaluate the efficacy and safety of long-term treatment with asfotase alfa in routine clinical practice
Survey method	All-case surveillance
Target patients	All patients treated with asfotase alfa[115]
Follow-up period	From the time of enrollment to the end of the survey period (up to 9 years)
Target number of patients	All patients treated with asfotase alfa[115]
Main survey items	Patient characteristics, use-status of asfotase alfa, concomitant drugs, safety evaluations (e.g., immunogenicity, injection-related reactions, serum calcium abnormalities, adverse events), efficacy evaluations (e.g., hypophosphatasia-related laboratory test values, history and assessment of skeletal disease, growth and development, survival duration)

III. Overall Evaluation

As a result of the above review, PMDA has concluded that asfotase alfa (Strensiq) may be approved for the indication and dosage and administration shown below, with the following conditions. Since asfotase alfa is designated as an orphan drug, the re-examination period is 10 years. The drug substance and the drug product are both classified as a powerful drug, and the product is classified as a biological product.

[Indication]

Hypophosphatasia

[Dosage and administration]

The usual dosage of Asfotase Alfa (Genetical Recombination) is 1 mg/kg administered subcutaneously 6 times weekly or 2 mg/kg administered subcutaneously 3 times weekly. The dose should be reduced appropriately according to the patient's condition.

[Conditions for approval]

The applicant is required to:

- Develop a risk management plan and implement it appropriately.
- Conduct a post-marketing drug use-results survey covering all patients treated with the product during the re-examination period to obtain characteristics of the patients because of the very limited number of Japanese patients included in the clinical studies, and collect data on the safety and efficacy of the product as soon as possible, so that appropriate measures can be taken to ensure its proper use.