Alkaline Phosphatase Replacement Therapy

10

Maria Luisa Bianchi and Silvia Vai

Abstract

Hypophosphatasia (HPP) is a rare genetic disease, characterized by the defective production of tissue-non-specific alkaline phosphatase (TNSALP). Six subtypes of the disease – affecting neonates (beginning in utero), infants, children, or adults – are recognized: perinatal lethal, prenatal benign, infantile, childhood, adult, and odontohypophosphatasia. The clinical presentation of these subtypes is very different and the severity ranges from mild to lethal. This chapter, after an overview of the genetics, epidemiology, classification, and clinical presentation of the different forms of HPP, will review the current experience with enzyme replacement therapy (ERT).

Keywords

Asfotase alfa · Alkaline phosphatase · Bone · Enzyme replacement therapy · Fractures · Hypophosphatasia · Hypomineralization · Teeth

Abbreviations

ALP	Alkaline phosphatase
BMD	Bone mineral density
BSALP	Bone-specific alkaline phosphatase
CPAP	Continuous positive airway pressure
DXA	Dual-energy X-ray absorptiometry
ERT	Enzyme replacement therapy
GABA	Gamma-aminobutyric acid
HPP	Hypophosphatasia
NPP1	Nucleoside pyrophosphohydrolase-1
NSAIDs	Non steroidal anti-inflammatory drugs
PEA	Phosphoethanolamine
PHOSPHO1	Phosphatase orphan 1
Pi	Inorganic phosphate
PL	Pyridoxal
PLP	Pyridoxal-5′-phosphate
PPi	Inorganic pyrophosphate
PTH	Parathyroid hormone
RGI-C	Radiographic global impression of change
TNSALP	Tissue-non-specific alkaline phosphatase

M. L. Bianchi (✉) · S. Vai
Experimental Laboratory for Children's Bone Metabolism Research, Bone Metabolism Unit, Istituto Auxologico Italiano IRCCS, Milan, Italy
e-mail: ml.bianchi@auxologico.it

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10.1 Hypophosphatasia

Hypophosphatasia (HPP), first described in 1948 (Rathbun 1948), is a rare inherited disorder of bone and mineral metabolism, caused by loss-of-function mutations in the *ALPL* gene, leading to reduced activity of the tissue-non-specific isoenzyme of alkaline phosphatase (TNSALP). Over 360 *ALPL* mutations are known.

The clinical presentation of HPP is very variable, depending on age of appearance and, possibly, on type of mutation and mechanism of inheritance. The most severe symptoms and signs are observed in neonates, infants and children, and a worse prognosis is usually associated with earlier manifestations. Defective bone and tooth mineralization, respiratory insufficiency, seizures, chronic pain, altered calcium and phosphate metabolism, renal problems, impaired growth and mobility, developmental delay, recurrent fractures, premature tooth loss are typical clinical findings. Biochemical tests show persistent hypophosphatasemia (low levels of serum alkaline phosphatase (ALP) and TNSALP, adjusted for age and gender) and increased levels of ALP substrates (inorganic pyrophosphate [PPi]; pyridoxal-5′-phosphate [PLP], the active metabolite of vitamin B6; and phosphoethanolamine [PEA]). Among them, PPi is a potent inhibitor of mineralization, and its accumulation explains the bone and tooth complications that are a hallmark of HPP.

The clinical classification is essentially dependent on age at onset of first signs and symptoms, and six subtypes of the disease are currently recognized, with severity ranging from mild to lethal (see Sect. 10.1.4) (Hofmann et al. 2013; Linglart and Biosse-Duplan 2016; Mornet 2018; Whyte 2016).

In the last decade, the efficacy and safety of enzyme replacement therapy with asfotase alfa, a recombinant TNSALP, has been demonstrated in newborns, infants, and children with severe HPP. In 2015, asfotase alfa has been approved by regulatory agencies in several countries, including the European Union (Hofmann et al. 2016; Mornet 2018; Whyte 2017a).

10.1.1 Alkaline Phosphatase

Alkaline phosphatase (ALP) is a membrane-bound phosphomonoesterase that catalyzes dephosphorylation reactions (cleavage of phosphoester bonds with release of a hydroxyl group and phosphate) and is essential in the catabolism of PPi and other substances. It is present in all animal species. In humans, four ALP isoenzymes, encoded by different genes, are known: three are tissue-specific ALPs (intestinal ALP, placental ALP, placenta-like or germ cell ALP), and the fourth is the ubiquitous non-specific TNSALP, that accounts for about 95% of the total serum ALP activity and is most abundant in liver, bone, and kidney (Caswell et al. 1991; Cole 2008; Mornet 2008; Hofmann et al. 2013; Hoshi et al. 1997; Whyte 2010). In bone, TNSALP is found in pre-osteoblasts, osteoblasts, matrix vesicles, osteoid in areas of new bone formation, trabecular lining cells, newly embedded osteocytes, subperiosteal, endosteal, and bone marrow cells. TNSALP is also present in growth plates (chondrocytes and cartilage matrix), articular cartilages and teeth (ameloblasts and odontoblasts) (Hoshi et al. 1997; Miao and Scutt 2002). TNSALP activity is essential for bone and teeth mineralization, and its deficiency leads to skeletal hypomineralization, a clinical hallmark of HPP. The bone TNSALP isoform is often called bone-specific ALP (BSALP). TNSALP may also have a role in inflammatory processes, as suggested by the occurrence of muscle, bone, and cartilage damage, including osteomyelitis and arthritis, in HPP (Hofmann et al. 2013).

TNSALP function in tissues other than bone is still incompletely known. Reviews of current knowledge are provided by Buchet et al. (2013) and Mornet (2018). TNSALP is certainly involved in the metabolism of vitamin B6, whose active metabolite (PLP) has a key role in several physiological processes, including the synthesis of major neurotransmitters (serotonin, dopamine, epinephrine, norepinephrine, GABA). Studies on knockout mice have suggested that the defective dephosphorylation of PLP to pyridoxal (PL) could explain the seizures observed in some peri-

natal/infantile HPP forms. Such seizures are the only known clinical sign of altered vitamin B6 metabolism in HPP, although, for unclear reasons, a positive response to vitamin B6 treatment in these cases seems to be an ominous sign. It must be underlined that such seizures are only observed in very young subjects. Older children or adults have no signs of vitamin B6 deficiency or toxicity (Baumgartner-Sigl et al. 2007; Mornet 2008; Whyte 2010).

TNSALP seems also involved in the development of central nervous system and in some brain functions (Fonta et al. 2005; Hofmann et al. 2013; Kermer et al. 2010), and these actions might explain the neurological and psychological signs and symptoms (neonatal seizures, chronic pain, anxiety, restlessness, depression) that are often observed in HPP.

10.1.2 Genetics

The *ALPL* gene is located on the short (p) arm of chromosome 1, bands 1p36.1-p34 (Weiss et al. 1988; Smith et al. 1988). Human TNSALP contains 524 aminoacid residues (Silvent et al. 2014). HPP is caused by any mutation in the *ALPL* gene leading to decreased TNSALP activity and increased levels of its substrates. As of August 2018, the *Tissue Nonspecific Alkaline Phosphatase Gene Mutations Database* (http://www.sesep.uvsq.fr/03_hypo_mutations.php) includes 365 *ALPL* mutations. The inheritance mechanism can be autosomal recessive or dominant. The most severe forms of HPP are autosomal recessive and are characterized by nearly total suppression of TNSALP activity. The milder forms can be either autosomal recessive or autosomal dominant and are characterized by reduced, but not suppressed, TNSALP activity. Some severe mutations may cause mild forms of HPP in heterozygotes, due to a dominant negative effect (Fauvert et al. 2009; Mornet and Nunes 2011; Mornet 2017). The phenotypic presentation of the disease may be significantly different even within the same family (Hofmann et al. 2014).

10.1.3 Epidemiology

HPP is present worldwide, but its prevalence in different populations is highly variable. Canadians have a particularly high prevalence of the severe form, estimated at 1:100,000 (Fraser 1957). In particular, Canadian Mennonites show a very high prevalence (up to 1/2500), and 1:25 subjects may be a carrier (Orton et al. 2008). In African-Americans, HPP seems to be particularly rare (Whyte et al. 2006). In Europe, the prevalence of severe HPP has been estimated at 1:300,000 (Mornet et al. 2011). In Japan, the prevalence of the perinatal lethal form is about 1:900,000 and seems often due to homozygosis for *ALPL* mutation c.1559delT, that is found only among the Japanese, with an estimated carrier frequency of 1:480 (Watanabe et al. 2011).

The prevalence of the milder forms of HPP, including the adult forms, is extremely difficult to estimate, as many cases probably remain undiagnosed. According to a genetic model proposed by Mornet et al., the prevalence of dominant mild HPP in the European population might be about 1:6370 (Mornet et al. 2011).

10.1.4 Classification

The clinical presentation of HPP is very variable, ranging from lethal perinatal forms (death in utero or soon after birth, with almost total absence of bone mineralization) to mild adult forms (dental problems and osteopenia) (Bianchi 2015).

The original classification of HPP, essentially unchanged since 1957, described five HPP subtypes: "perinatal lethal HPP", "infantile HPP", "childhood HPP", "adult HPP", and "odonto-HPP". A sixth subtype "prenatal (or perinatal) benign HPP" was subsequently identified and added (Wenkert et al. 2011).

In 2015, reappraising their 25-year experience with 173 pediatric HPP patients, Whyte et al. proposed to distinguish two forms of "childhood HPP", mild and severe (Whyte et al. 2015). Accordingly, an updated classification would now recognize seven "major forms" of HPP, that

is, in order of increasing severity: "odonto HPP", "adult HPP", "mild childhood HPP", "severe childhood HPP", "infantile HPP", "perinatal HPP", plus the time-limited form "prenatal benign HPP" (Whyte 2017a) (Table 10.1).

In practice, there can be considerable overlapping of clinical features, particularly in the infantile and childhood forms, and some cases can be very difficult to classify. Both children and adults can present with pain, stiffness, and weakness of the lower limbs (possibly due to bone microfractures). Also, anomalies of tooth color, eruption and exfoliation, shape and structure, enamel, dentin, cementum are observed in both childhood and adult forms. The clinical severity of HPP is essentially linked to its skeletal complications, and a worse prognosis is usually associated with earlier signs and symptoms (Cole 2008; Fallon et al. 1984; Hofmann et al. 2013; Whyte et al. 2015; Whyte 2017a).

Very recently, Whyte et al. (2018), re-evaluating a cohort of 165 preteenager HPP patients, highlighted the four biochemical hallmarks of HPP in children. All patients had subnormal serum total and bone-specific ALP, and elevated plasma PLP, and nearly all had high urinary PPi excretion. The mean levels of these four biomarkers correlated with HPP severity, ranked according to the proposed HPP nosology ("odonto HPP", "mild childhood HPP", "severe childhood HPP", "infantile HPP"), with data overlapping among the four patient groups. The authors conclude that "these four biochemical hallmarks represent both a sensitive and reliable tool for diagnosing children with HPP", and "validate our expanded clinical nosology for pediatric HPP" as an "improved framework for conceptualizing and working with this disorder's remarkably broad-ranging severity".

10.1.5 Bone and Dental Problems in HPP

Defective mineralization of bone and teeth is the most characteristic and severe consequence of insufficient TNSALP activity (Figs. 10.1, 10.2, and 10.3) (Hofmann et al. 2013; Linglart and Biosse-Duplan 2016; Whyte 2017a). The mineralization of cartilage, bone, and teeth is the result of physicochemical and biochemical processes regulated by several promoting and inhibiting factors. Among them, three phosphatases – TNSALP, phosphatase orphan 1 (PHOSPHO1), and nucleoside pyrophosphohydrolase-1 (NPP1) – play an essential role in maintaining an optimal Pi/PPi ratio. This ratio is crucial in the mineralization process, where Pi is needed for the formation of hydroxyapatite crystals, while PPi inhibits it (Millán 2013).

In an early phase of mineralization, Ca^{2+} and Pi accumulate within the bone matrix vesicles, forming hydroxyapatite crystals. In a second phase, the vesicles' membranes break down and the hydroxyapatite crystals are deposited in the collagenous matrix (Anderson 1995; Golub 2009; Hessle et al. 2002; Schinke et al. 1999).

In HPP, insufficient TNSALP activity leads to the accumulation of excess PPi, which inhibits the calcium/phosphate crystal nucleation and hampers the growth of hydroxyapatite crystals in the matrix vesicles (Hessle et al. 2002; Harmey et al. 2004; Anderson et al. 2004; Orimo 2010). Even if osteoblasts seem to be unaffected (Wennberg et al. 2000), the whole process of bone mineralization is seriously impaired, and hypomineralized bone, spontaneous fractures, tooth loss, as well as soft tissue calcifications (osteoarthritis, arterial calcification), are characteristic manifestations of the disease (Millán 2013).

Regarding dental problems, premature exfoliation and structural alterations of the primary (deciduous) teeth and an increased occurrence of severe dental caries is observed in all forms of HPP (Fig. 10.2). Alveolar bone and dental cementum are poorly mineralized, and premature tooth loss is common. The anterior teeth (incisors) are more frequently affected. Impaired dentinogenesis, revealed by large pulp chambers, and enamel hypoplasia, predisposing to dental caries, are also described. The primary teeth are most often affected, but permanent teeth are not spared (Atar and Körperich 2010; Beumer et al. 1973; Olsson et al. 1996; van den Bos et al. 2005).

Table 10.1 Different forms of hypophosphatasia

Form	Inheritance	Clinical course and features	Dental features
Perinatal lethal	AR	Most severe form	–
		Stillbirth or death within days/weeks after birth	
		Severe hypomineralization (deformed and short limbs)	
		Derangements in calcium/phosphate metabolism	
		Osteochondral spurs in forearms, legs	
		Severe lung hypoplasia (chest deformities, rib fractures)	
		Seizures, dependent by pyridoxine (B6)	
Prenatal (or perinatal) benign	AR/AD	Benign evolution (but long-term course unknown)	–
		Limb shortening and bowing of long bones observed in utero	
		Spontaneous improvement of skeletal defects after birth	
Infantile	AR	First symptoms during the first 6 months of life	Premature loss of deciduous teeth
		Poor prognosis during the 1st year of life (mortality has been estimated to be 50% during infancy)	
		Severe hypomineralization (rachitic ribs)	
		Premature craniosynostosis (Chiari I malformation, hydrostatic hydrocephalus, hydrosyringomyelia)	
		Rickets-like signs of hypomineralization	
		Swallowing disorders, irritability, seizures	
		Severe muscular hypotonia	
		Hypercalciuria, nephrocalcinosis	
Childhood ("mild" or "severe")	AR/AD	First symptoms after 6th month of life	Premature loss of deciduous teeth
		Rickets-like signs of hypomineralization	
		Short stature, failure to thrive	Caries
		Delayed walking	
		Repeated fractures	
		Waddling gait due to bone deformities	
		Chronic bone pain (lower extremities)	
		Muscular hypotonia	
		Lack of appetite, nausea, gastrointestinal problems	
Adult	AR/AD	Presents in middle age	Loss of permanent teeth at 40–60 years of age
		Stress fractures of metatarsals, tibia. Femur pseudofractures	
		Fragility fractures	
		Osteomalacia, osteoporosis	
		History of delayed fracture healing in childhood; often mild rickets	
		Chondrocalcinosis, osteoarthritis	
		Myopathy, weakness	
		Renal abnormalities, reduced GFR nephrocalcinosis and kidney stones	
		Psychiatric symptoms (insomnia, restlessness, anxiety, depression)	
Odonto HPP	AR/AD	Not associated with bone, articular, or muscular problems	Premature (i.e. age <5 years) exfoliation of deciduous and/or permanent teeth (mainly incisors)
			Severe dental caries
			Reduced thickness of dentin Enlarged pulp chambers
			Alveolar bone loss

Legend: *AD* autosomal dominant, *AR* autosomal recessive, *GFR* glomerular filtration rate

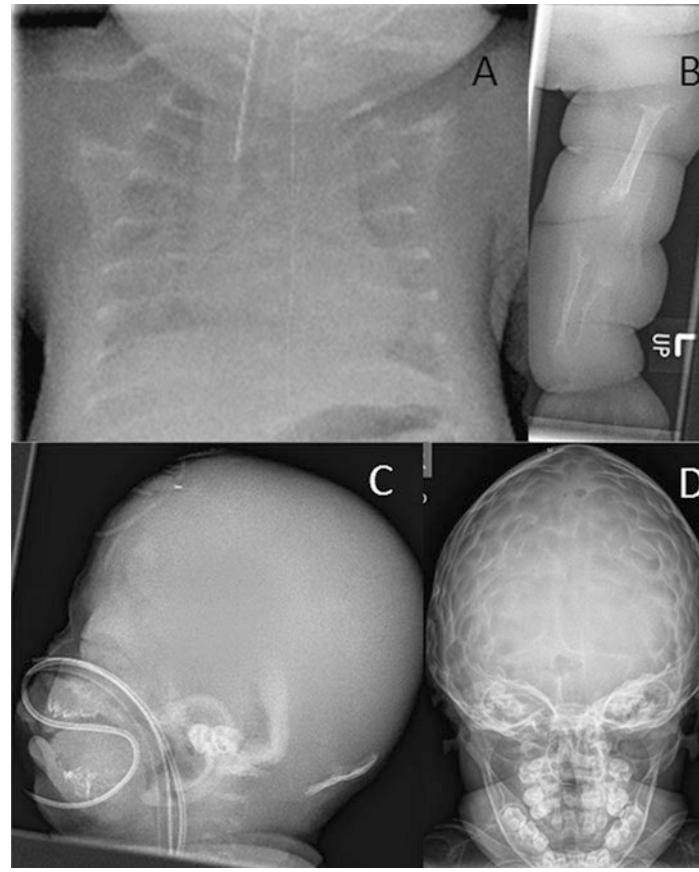

Fig. 10.1 Radiographies of a case of perinatal and infantile HPP. (**a**) Chest of an infant with thin ribs, lung hypoplasia, endotracheal intubation and ventilation. (**b**) Upper limb showing extreme hypomineralization. (**c**) Lateral skull showing severe hypomineralization, and ossification only at frontal bone, base and occiput. (**d**) Antero-posterior skull showing a copper beaten skull due to craniosynostosis with increased intracranial pressure. (From Hofmann et al. 2013, reproduced with permission)

10.1.6 Diagnosis

The diagnosis of HPP must be based on a consistent set of medical history and physical examination data, biochemical tests, and skeletal radiographic findings (that can be pathognomonic in perinatal, infantile, and severe childhood HPP) (Bianchi 2015; Hofmann et al. 2013; Kishnani et al. 2017; Linglart and Biosse-Duplan 2016; Whyte 2016, 2017a, b). As all rare diseases, HPP (the mild forms in particular) can be difficult to recognize and the diagnosis is often delayed. HPP is associated with a very wide range of presenting signs, symptoms, and complications (defective skeletal mineralization, respiratory insufficiency, seizures, altered calcium and phosphate metabolism, renal problems, pain, delay in growth and development, impaired mobility, recurrent fractures, tooth loss, etc.), that can be misinterpreted as indicators of other more common diseases. The clinical findings, and consequently the differential diagnosis, are very different depending on age at presentation. For example, the presence of short bowed legs revealed by ultrasonography in a fetus might suggest osteogenesis imperfecta or other skeletal dysplasias. In a neonate, HPP should be differentiated from congenital rickets, neonatal hyperparathyroidism, osteogenesis imperfecta, mucolipidosis II. In an infant, HPP might be misinterpreted as any cause of failure to thrive, nutritional rickets, or nephrocalcinosis due to idiopathic hypercalcemia. In a child, the presence of short stature, bone pain, fractures, motor delay might suggest mild osteogenesis imperfecta, myopathies, arthritis, chronic recurrent multifocal osteomyelitis, fibrous dysplasia. In an adult, especially in the presence of fractures, HPP can easily be confused with osteoporosis (Bishop 2015; Hofmann et al. 2013; Linglart and Biosse-Duplan 2016).

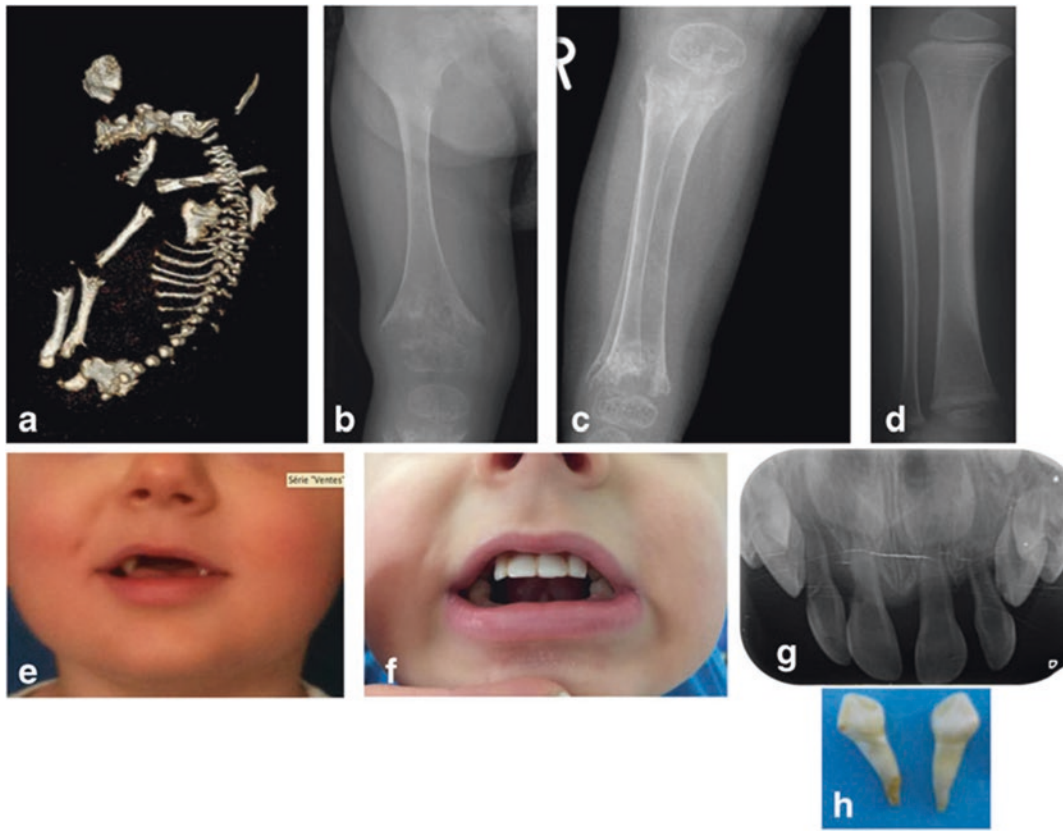

Fig. 10.2 Images showing clinical characteristics of HPP in children. (**a**) Scannographic image at 30 weeks of pregnancy in a fetus affected with hypophosphatasia and a homozygous mutation in the ALPL gene. Note the lack of mineralization of the skull, the thin ribs, but most of all, the abnormal distal femoral metaphyses with eperons and irregularities. (**b**) Femur and (**c**) tibia X-rays in an 18-month-young boy with HPP with a compound heterozygous mutation in the ALPL gene. (**d**) For comparison, an X-ray of an unaffected 20-month-old boy is shown. (**e**) Premature loss of the anterior upper and lower teeth before the age of 2 years. (**f**) Anicesmile with a fixed partial denture in the same child 1 year later. (**g**) Retroalveolar radiograph of the same patient before he lost upper incisors and canines during the following year. Note the reduced alveolar bone level around the incisors. (**h**) Pictures of exfoliated teeth (decidual canines) with their entire roots. (From Linglart and Biosse-Duplan 2016, reproduced with permission)

The typical laboratory findings of HPP are persistently low serum levels of ALP for age and gender, and increased levels of its substrates PPi, PLP, and PEA. However, low ALP activity alone, even if correctly based on sex- and age-specific reference ranges, cannot establish a diagnosis of HPP, as it may depend on many different causes (use of certain drugs, celiac disease, hypothyroidism, osteogenesis imperfecta type II, milk-alkali syndrome, vitamin D toxicity, etc.). High serum levels of PLP are a more specific marker of HPP, and are usually correlated with disease severity (Whyte 2016). It can also be noted at this point that a standardized assay for serum PPi, a potentially very useful laboratory test in the diagnosis and follow-up of HPP, is currently not available.

Genetic analysis of *TNSALP* mutations is often unnecessary for a diagnosis of HPP, but is obviously helpful to clarify inheritance patterns, evaluate the risk of HPP recurrence in a future pregnancy, and for prenatal assessment. However, even a positive mutation analysis is not sufficient for a diagnosis of HPP, if not accompanied by clinical, biochemical and radiologic evidence of disease (Whyte 2017a).

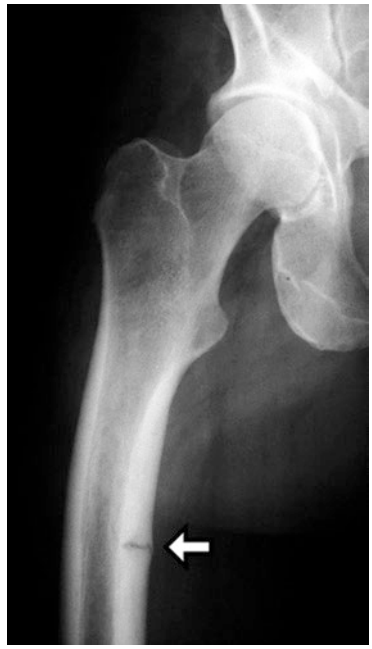

Fig. 10.3 HPP in an adult with severe childhood type. X-ray shows a pseudofrature (arrow) medially in the proximal femur who presented also bowing and cortical thickening. (From Whyte 2017a, reproduced with permission)

Whyte also underlines the importance of a correct diagnosis of HPP before making the decision to treat a patient with asfotase alfa, because mistakenly using the drug in conditions other than HPP could result in excessive mineralization with negative consequences (Whyte 2017a). In an adult, the misdiagnosis of HPP as osteoporosis may lead to the erroneous prescription of a bisphosphonate, which is contraindicated in HPP (Wüster and Ziegler 1992; Sutton et al. 2012; Whyte 2009).

10.2 General Management of HPP

Until the availability of enzyme replacement therapy (ERT, see below), therapeutic interventions in HPP addressed its different clinical problems (chronic pain, musculo-articular-skeletal alterations, fractures, psychological and neurological problems, renal problems, dental problems), aiming to alleviate symptoms and prevent complications (Rockman-Greenberg 2013). The wide spectrum of HPP manifestations ideally required the collaboration of an experienced multispecialty team.

10.2.1 Nutrition

In the presence of hyperphosphatemia, restriction of dietary phosphate (Pi) and/or pharmacologic binding of dietary Pi have been proposed, since Pi is known to inhibit TNSALP activity and gene expression (Wenkert et al. 2002).

In children, adequate nutrition should be ensured, especially in the presence of growth retardation. Vitamin D insufficiency should be corrected with standard supplementation, taking care to avoid too high doses that could aggravate the hypercalcemia/hypercalciuria in infantile HPP (Mornet and Nunes 2011). Active metabolites of vitamin D (such as 1α-hydroxy-vitamin D or 1,25-dihydroxyvitamin D) should not be used, to avoid increase of intestinal Pi absorption and serum Pi levels. Calcium supplementation is also to be avoided except in carefully selected cases, considering the risk of hypercalcemia and kidney stones. In adults, supplementation with calcium and vitamin D can be used to prevent secondary hyperparathyroidism (Mornet and Nunes 2011).

10.2.2 Physical Activity

Moderate but regular physical exercise is recommended at any age, first to build and then to maintain bone mineral mass and bone strength. Only low-impact physical activities are suitable, especially in the severe forms of HPP, to minimize the risk of fragility fractures.

10.2.3 Inflammatory Manifestations

Patients with bone and joint pain and inflammatory symptoms may benefit from the judicious intermittent use of non steroidal anti-inflammatory drugs (NSAIDs), with significant subjective and functional improvement, that may last for some weeks after withdrawal. NSAIDs

have also been successfully used for bone and joint pain in childhood HPP (Girschick et al. 2006). In the presence of impaired renal function, NSAID use is risky and requires close monitoring (Hofmann et al. 2013).

10.2.4 Osteoporosis and Fractures

Osteoporosis and bone fragility are a major problem in the severe forms of HPP. The prevention of fractures through the avoidance of risky physical activities is very important, since fractures in HPP patients heal with great difficulty and in a long time, requiring prolonged casting or even orthopedic interventions.

Bisphosphonates, the classical anti-osteoporosis drugs, have not been seriously studied in HPP (Mornet and Nunes 2011). There are theoretical reasons against them, however, as they are structurally related to PPi and can further suppress TNSALP activity (Wüster and Ziegler 1992). Moreover, there is a major difference between the bone fragility of HPP and that of classical osteoporosis. In HPP, bone fragility is caused by deficient bone mineralization, while in osteoporosis it derives from excess bone resorption. For this reason, the anti-resorptive drugs like bisphosphonates and denosumab that are so effective in classical osteoporosis are possibly contraindicated in HPP and may have untoward side effects. It has been reported that adults with undiagnosed HPP treated with bisphosphonates may have an increase in and/or worsening of fractures, or may sustain atypical (lateral subtrochanteric) femur fractures (Fig. 10.4) (Cundy et al. 2015; Mornet and Nunes 2011; Sutton et al. 2012; Whyte 2009).

Since PTH stimulates TNSALP synthesis in osteoblasts, teriparatide (recombinant human parathyroid hormone 1–34) has been used in some adults with HPP. Whyte et al. (2007) reported the healing of metatarsal stress fractures and proximal femur pseudofractures with a 18-month teriparatide treatment. Camacho et al. (2008) reported the positive effects of a 24-month treatment in a 75-year-old woman with multiple low-trauma fractures: skeletal mineralization and bone turnover markers improved, and there were no more fractures. Doshi et al. (2009) described an interesting case of bisphosphonate-related atypical femur fractures in a 50-year-old woman, successfully treated with teriparatide for 16 months after the correct diagnosis of HPP was

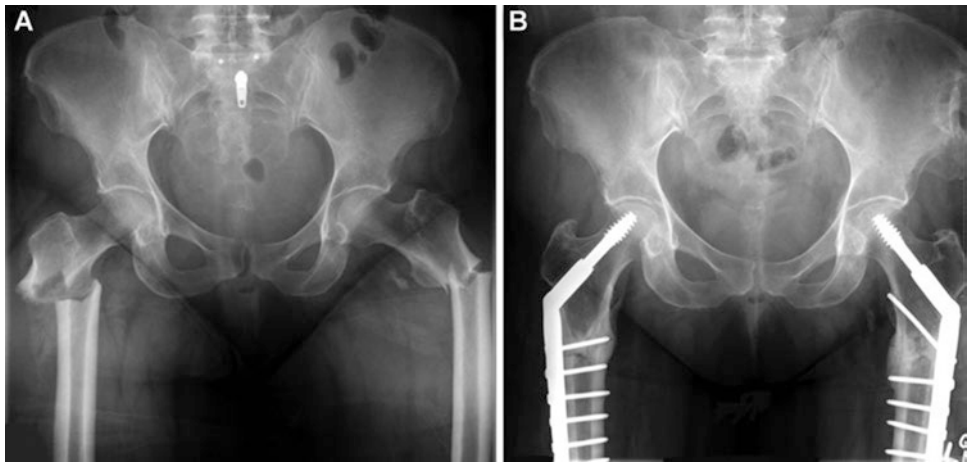

Fig. 10.4 X-rays images in an adult with HPP and treated with bisphosphonates (BP) (**a**) Bilateral acute subtrochanteric femoral fractures occurred in June 2010 after approximately 4 years of BP exposure. Note also the cortical thickening of both upper femoral shafts. (**b**) In August 2011, approximately 18 months after cessation of BP exposure, there was persisting thigh pain but evidence of fracture healing with callus formation although the cortical fracture lines can still be seen bilaterally. (From Sutton et al. 2012, reproduced with permission)

made. There are other case reports of successful treatment (Schalin-Jäntti et al. 2010; Camacho et al. 2016), but also cases in which the benefits did not last (Gagnon et al. 2010), or no significant effect was observed (Laroche 2012). Considering these inconsistent results, Gagnon et al. (2010) suggested that the response might depend on the specific gene mutations. Teriparatide should not be used for a total duration of more than 2 years in a single patient, and must not be used in young patients with open epiphyses, due to a potentially increased risk of osteosarcoma (observed in animal studies) (Mornet and Nunes 2011).

Calcitonin followed by a bisphosphonate (clodronate) was unsuccessfully tried in a 7-month-old girl with infantile HPP (Deeb et al. 2000).

Anti-sclerostin treatment might stimulate bone formation, and there is a report that eight adult patients with HPP showed increases in bone formation markers and bone mineral density (Seefried et al. 2017).

Bone marrow and stem cell transplantation has been tried in some infants and children with life-threatening HPP, with some improvement in skeletal mineralization and prolonged survival (until 3–7 years), but without consistent improvement in ALP activity (Cahill et al. 2007; Taketani et al. 2015; Whyte et al. 2003).

10.2.5 Neurological and Psychological Symptoms

Seizures, apparently due to defective dephosphorylation of PLP, are reported in severe perinatal and infantile HPP. For unclear reasons, their response to vitamin B6 or pyridoxine treatment seems not a positive sign and may on the contrary suggest a lethal prognosis (Baumgartner-Sigl et al. 2007; Mornet 2008; Whyte 2010).

Chronic pain, insomnia, anxiety, irritability, restlessness, depression can be very disturbing symptoms in the less severe forms of HPP and should be appropriately treated. Recent progress in the elucidation of TNSALP activity in nociception and purinergic signaling might lead to more specific approaches (Hofmann et al. 2013).

10.2.6 Neurosurgical Problems

In the infantile and childhood forms, the premature fusion of cranial sutures is common, and radiological, neurological, and ophtalmoscopic assessments should be regularly made until adolescence. Complications like secondary ectopia of cerebellar tonsils (Chiari I malformation) or hydrosyringomyelia may appear, and in these cases lifetime surveillance is needed (Collmann et al. 2009). Neurosurgical interventions may be required in the presence of neurologic signs and symptoms of increased intracranial pressure (headache, seizures, papilledema, numbness of extremities, paralysis).

10.2.7 Renal Problems

Kidneys can be involved in HPP in several ways. High arterial pressure is common and must be treated. Kidney stones can be a complication of hypercalcemia and hypercalciuria. Hyperphosphatemia may require treatment with phosphate binders. Nephrocalcinosis can also develop, with progressive worsening of renal function. The prudent, short-term use of NSAIDs might be helpful, possibly with the supervision of a nephrologist (Rockman-Greenberg 2013).

10.3 Enzyme Replacement Therapy with Asfotase Alfa

The first attempts at ERT were made with intravenous administration of ALP-enriched plasma from patients with Paget bone disease, purified human liver ALP, or purified human placental ALP in infants with severe HPP, but the results were disappointing (Weninger et al. 1989; Whyte et al. 1982, 1992). Some years later, transplantation of T-cell-depleted bone marrow (Whyte et al. 2003), bone fragments and cultured osteoblasts (Cahill et al. 2007), or mesenchymal stem-cells (Tadokoro et al. 2009; Taketani et al. 2013, 2015) resulted in encouraging, although limited, improvement. These first studies suggested that

increasing enzyme activity in plasma was not sufficient, while TNSALP-expressing bone cells could help correct the genetic defect of HPP.

In 2004, Enobia Pharma Inc. (a biotech company based in Montreal, Canada) filed a patent application for a bioengineered substitute of TNSALP (original documentation available at https://patentimages.storage.googleapis.com/cf/e0/bd/7ecc5788ba1853/US7763712.pdf). This product (sALP-FcD10, first named ENB-0040; currently called asfotase alfa) is a recombinant fusion glycoprotein made of two identical polypeptide chains (connected by disulfide bonds), that contain the catalytic ectodomain of human TNSALP (i.e. the soluble part of TNSALP, cut at the C-terminal membrane-bound region), with the addition of the human IgG1 Fc region and a C-terminal deca-aspartate motif. The Fc region was added to facilitate cromatographic purification of the drug, and the deca-aspartate motif, which has high affinity for hydroxyapatite crystals, for specific bone targeting (Fig. 10.5) (Hofmann et al. 2016).

In 2011, Enobia Pharma was acquired by Alexion Pharmaceuticals Inc. (New Haven, CT, USA), and ENB-0040 was renamed asfotase alfa. Since 2008, ENB-0040/asfotase alfa has been investigated in many studies, first in animals and then in children with severe HPP. The positive results led to its approval by the regulatory agencies of Japan (PMDA), European Union (EMA), Canada (Health Canada), and USA (FDA) in 2015. Asfotase alfa, currently marketed as Strensiq® by Alexion Pharmaceuticals, is currently the only approved treatment for HPP, with the indication "*treatment of patients with perinatal-, infantile- and juvenile-onset hypophosphatasia*". The European Medicines Agency product information is available online (Strensiq® EMA product information 2018).

Strensiq® (asfotase alfa) is a clear, colorless, aqueous solution for subcutaneous injections, now available in single use vials at different doses and concentrations (18 mg/0.45 mL, 28 mg/0.7 mL, 40 mg/1.0 mL, 80 mg/0.8 mL). The recommended dosage is 2 mg/kg body weight three times a week or 1 mg/kg body weight six times a week. The maximum injection volume should not exceed 1 mL, and if necessary, multiple subcutaneous injections can be administered at the same time. The safety and efficacy of asfotase alfa in HPP patients with renal or hepatic problems have not been studied (Hofmann et al. 2016; Whyte 2017a; see also Strensiq® Alexion product information 2018; Strensiq® Alexion Prescription Information 2018). Table 10.2 resumes the clinical trials on HPP treatment with asfotase alfa, as listed at *https://clinicaltrials.gov*.

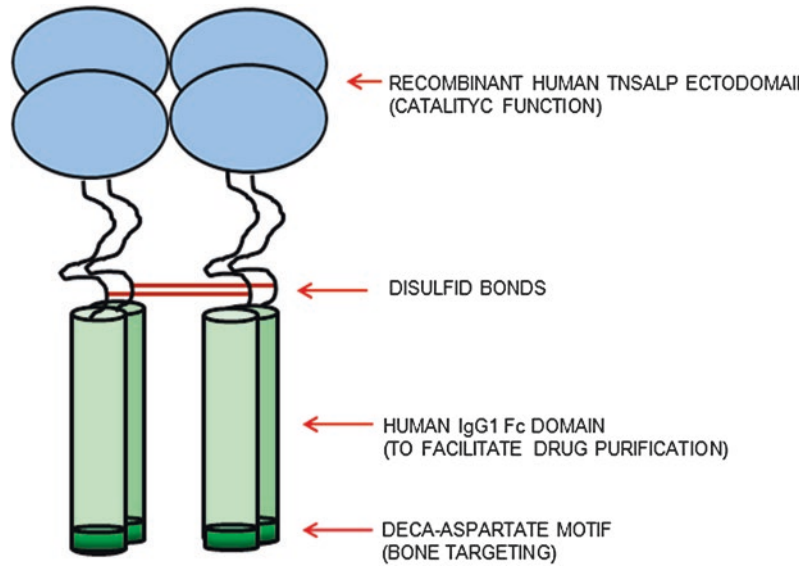

Fig. 10.5 The structure of asfotase alfa (drawn as a homotetramer): a recombinant fusion glycoprotein, containing the human TNSALP ectodomain with the addition of the human IgG1 Fc region and a C-terminal deca-aspartate motif. (Redrawn from http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/003794/WC500194340.pdf)

Table 10.2 Clinical trials on hypophosphatasia and asfotase alfa

NCT number	Titles	Recruitment	Characteristics	Population	Sponsor	Dates and Results	Locations
NCT03418389	Evaluate and monitor physical performance of adults treated with asfotase alfa for HPP	Not yet recruiting	Observational	Enrollment:	Alexion Pharm.	Start: July 2018	Würzburg University Hospital, Würzburg, Germany
			Cohort time				
			Perspective	Age: 18 years and older (adult, older adult)		First posted: Feb. 1 2018	
			Outcome measures:				
			Physical performance				
			Body composition	Sex: M and F		Last update posted: May 1, 2018	
			Health related quality of life				
			And 4 more outcomes				
NCT02797821	Pharmacokinetic, and dose response study of asfotase alfa in adult patients with pediatric-onset HPP	Completed	Interventional phase: 2	Enrollment: 27	Alexion Pharm.	Study start: Jun. 2016	Shriners Hospitals for Children, Saint Louis, MO, USA
			Randomized	Age: 18 years and older (adult, older adult)		First posted: Jun. 14, 2016	Duke University Medical Center, Durham, NC, USA
			Parallel assignment				
			Masking: none (open label)	Sex: M and F		Last update posted: Jul. 10, 2018	Vanderbilt Medical Center, Nashville, TN, USA
			Primary purpose: treatment				
			Outcome measures:			RESULTS not yet available	University of Würzburg, Würzburg, Germany
			Change in PPi from baseline to pre-3rd dose in week 9				
			Change in PLP from baseline to pre-3rd dose in week 9				
			And 11 more outcomes				

NCT02496689	Expanded access program for asfotase alfa treatment for patients with infantile- or juvenile-onset HPP	Approved for marketing	Expanded access	Enrollment: Age: child, adult, older adult Sex: M and F	Alexion Pharm.	Study start: NA First posted: Jul. 14, 2015 Last update posted: Oct. 25, 2017	Colorado Center for Bone Research, Lakewood, CO, USA Children's Hospital of Pittsburg, Pittsburg, PA, USA Hôpital Bicêtre, Le Kremlin- Bicêtre, France And 2 more
NCT02456038	Safety and efficacy of asfotase alfa in patients with HPP	Completed	Interventional phase 2 Single group assignment Masking: none (open label) Primary purpose: treatment Outcome measures: Number of subjects AEs as an assessment of safety of repeated SC injections of asfotase alfa Overall survival Effect of asfotase alfa on skeletal manifestations of HPP as measured by X-rays RGI-C scale And 4 more outcomes	Enrollment: [13] Age: child, Adult, older adult Sex: M and F	Translational Research Center for Medical Innovation, Kobe, Hyogo, Japan Osaka University Graduate School of Medicine, Osaka, Japan	Study start: August 2014 First posted: May 28, 2015 Last update posted: March 31, 2016 *RESULTS* Improved skeletal, respiratory and clinical symptoms No serious AEs (Kitaoka et al. 2017)	Kurume University Hospital, Kurume, Fukuoka, Japan National Hospital Organization, Nagara Medical Center, Gifu, Japan Hiroshima University Hospital, Hiroshima, Japan And 9 more

(continued)

Table 10.2 (continued)

NCT number	Titles	Recruitment	Characteristics	Population	Sponsor	Dates and Results	Locations
NCT01205152 (ENB-003-08)	Extension study of protocol ENB-002-08 (NCT00744042) – study of asfotase alfa in infants and young children with HPP	Completed	Interventional phase 2	Enrollment: 10	Alexion Pharm.	Study start: Apr. 2009	DuPont Hospital for Children, Wilmington, DE, USA
			Single group assignment	Age: 24 weeks to 42 months (child)		First posted: Sept. 20, 2010	St. John's Medical Research Institute, Springfield, MO, USA
			Masking: none (open label)	Sex: M and F		Last update posted: Nov.17, 2017	University of Nebraska Medical Center, Omaha, NE, USA
			Primary purpose: treatment			*RESULTS*	And 5 more
			Outcome measures:			Asfotase alfa mineralizes skeleton, including the ribs, improves respiratory function and survival in life-threatening perinatal and infantile HPP (Whyte et. al. 2016a, b; Whyte 2017a)	
			Long-term tolerability of SC asfotase alfa				
			Long-term efficacy of asfotase alfa in treating rickets in infants and young children				
			Long-term pharmacodynamics of asfotase alfa: plasma PPi and PLP levels				
			And 4 more outcomes				

NCT01203826	Extension study of protocol ENB-006-09 (NCT00952484) – Study of asfotase alfa in children with HPP	Completed	Interventional Phase: phase 2 Single group assignment Masking: none (open label) Primary purpose: treatment Outcome measures: Skeletal radiograph RGI-C scale compared to baseline (pre-treatment) in study ENB-006-09	Enrollment: 12 Age: 5–12 years (child) Sex: M and F	Alexion Pharm.	Study start: Apr. 2010 First posted: Sept. 16, 2010 Last update posted: Nov. 9, 2017 *RESULTS* With asfotase alfa significant X-rays and significant growth, strength, motor function, agility, and quality of life improvements, reduction in pain and disability (Whyte et al. 2016a)	Shriners Hospital for Children, Saint Louis, MO, USA Children's Hospital Health Sciences Centre, Winnipeg, Manitoba, Canada

(continued)

Table 10.2 (continued)

NCT number	Titles	Recruitment	Characteristics	Population	Sponsor	Dates and Results	Locations
NCT01176266 (ENB-010-10)	Open-label study of asfotase alfa in infants and children ≤5 years of age with HPP	Completed	Interventional	Enrollment: 69	Alexion Pharm.	Study start: Jul. 2010	Children's Hospital & Research Center Oakland, Oakland, CA, USA
			Phase 2 and 3	Age: up to 5 years (child)		First posted: Aug 5, 2010	
			Single group assignment	Sex: M and F		Last update posted: Feb 26, 2018	Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA
			Masking: none (open label)				
			Primary purpose: treatment				
			Outcome measures:			*RESULTS*	Children's Hospital of Pittsburg, Pittsburg, PE, USA
			Effect of asfotase alfa on skeletal manifestations of HPP			Improved survival rates (Whyte et al. 2016b)	
			Safety and tolerability of repeated SC injections of asfotase alfa				And 20 more
			Effect of asfotase alfa on ventilator-free survival (week 312)				
			And 11 more outcomes				
NCT01163149	Safety and efficacy study of asfotase alfa in adolescents and adults with HPP	Completed	Interventional phase 2	Enrollment: 19	Alexion Pharm.	Study start: Jun. 2010	Shriner's Hospital for Children, Saint Louis, MO, USA
			Randomized	Age: 13–65 years (child, adult, older adult)		First posted: Sept. 18, 2017	Duke University Medical Center, Durham, NC, USA
			Parallel assignment			Last update posted: Sept. 18, 2017	
			Masking: none (open label)				
			Primary purpose: treatment	Sex: M and F		RESULTS unpublished	Health Sciences Centre, Winnipeg University, Winnipeg, Manitoba, Canada
			Outcome measures:				
			Change from baseline to week 24 for plasma PLP and PPi				
			Safety and tolerability of asfotase alfa				
			And 6 more outcomes				

NCT00952484 (ENB 006-09)	Safety and efficacy of asfotase alfa in juvenile patients with HPP	Completed	Interventional phase 2	Enrollment: 13	Alexion Pharm.	Study start: Sept. 2009	Shriners Hospital for Children, St. Louis, MO, USA
			Randomized	Age: 5–12 years (child)		First posted: Aug. 6, 2009	The University of Manitoba Health Services Centre, Winnipeg, Manitoba, Canada
			Parallel assignment			Last update posted: May 2, 2016	
			Masking: none (open label)				
			Primary purpose: treatment	Sex: M and F		*RESULTS*	
			Outcome measures:			With asfotase alfa significant X-rays and significant growth, strength, motor function, agility, and quality of life improvements, reduction in pain and disability (Whyte et al. 2016a)	
			Change in rickets severity on skeletal radiographs from baseline to week 24 measured by RGI-C scale				
			Change in osteomalacia: osteoid thickness, osteoid volume/bone volume and mineralization lag time, measured by trans-iliac crest bone biopsy				
			And 8 more outcomes				

(continued)

Table 10.2 (continued)

NCT number	Titles	Recruitment	Characteristics	Population	Sponsor	Dates and Results	Locations
NCT00744042 (ENB002-08)	Safety and efficacy study of asfotase alfa in severely affected infants with HPP	Completed	Interventional phase 1 and 2	Enrollment: 11	Alexion Pharm.	Study start: Sept. 2008	Arkansas Children's Hospital, Little Rock, AR, USA
			Single group assignment	Age: up to 36 months (child)		First posted: Aug. 29, 2008	Du Pont Hospital for Children, Wilmington, DE, USA
			Masking: none (open label)	Sex: M and F		Last update posted: Feb. 22, 2016	St. John's Hospital, Springfield, MO, USA
			Primary purpose: treatment			*RESULTS*	And 7 more
			Outcome measures:			Improved skeletal mineralization, respiratory and physical function (Whyte et al. 2012), improved survival rates (Whyte et al. 2016a, b; White 2017a) (including extension NCT01205152)	
			Change in rickets severity from baseline to week 24 based on change in RGI-C				
			Maximum serum concentration of asfotase alfa (Cmax)				
			Time at maximum serum concentration of asfotase alfa (Tmax)				
			Area Under serum concentration-time curve to last measurable concentration of asfotase alfa (AUCt)				

NCT00739505	Safety study of human recombinant tissue non-specific alkaline phosphatase fusion protein asfotase alfa in adults with HPP adults with HPP	Completed	Interventional phase 1	Enrollment: 6	Alexion Pharm.	Study start: Aug. 2008	Barnes Jewish Hospital-Washington University School of Medicine, St. Louis, MO, USA
			Non-randomized	Age: 18–80 years (adult, older adult)		First posted: Aug. 21, 2008	
			Single group assignment				
			Masking: none (open label)			Last update posted: May 29, 2014	Duke University Medical Center, Durham, NC, USA
			Primary purpose: treatment				
			Outcome measures:	Sex: M and F		RESULTS unpublished	Department of Pediatrics & Child Health Sciences Centre Winnipeg University of Manitoba, Winnipeg, Manitoba, Canada
			Safety and tolerability of asfotase alfa given intravenously (IV) and given SC				
			Pharmacokinetics (PK) of asfotase alfa given IV and SC to assess bioavailability of the SC asfotase alfa				
NTC02531867	Post-approval clinical study of asfotase alfa treatment for patients with HPP in Japan	Completed	Interventional	Enrollment: 13	Alexion Pharm.	Study start: June 2015	
			Phase 4				
			Single group assignment	Age: child, adult, older adult		First posted: May 6, 2015	
			Masking: none (open label)				
			Primary purpose: treatment	Sex: M and F		Last update posted: May 8, 2017	
			Outcome measures:			RESULTS unpublished	
			Number of participants with AEs including injection site reactions (ISRs) and injection associated reactions (IARs)				

(continued)

Table 10.2 (continued)

NCT number	Titles	Recruitment	Characteristics	Population	Sponsor	Dates and Results	Locations
NCT00894075	Safety and efficacy study of ENB-0040 in juvenile patients with HPP	Withdrawn	Interventional	Enrollment: 0	Alexion Pharm.	Study start: July 2019	
			Phase 2	Age: 5–12 years (child)		First posted: Aug. 25, 2015	
			Single group assignment			Last update posted: Jan. 25 2013	
			Masking: none (open label)	Sex: M and F			
			Primary purpose: treatment				
			Outcome measures:				
			Skeletal radiographs using CGI-C scoring system				
			PK using serum peak and trough levels and PD of PPi and PLP as biomarkers for HPP				
NCT02306720	An observational, longitudinal, prospective, long-term registry of patients with HPP	Enrolling by invitation	Observational	Enrollment: 500	Alexion Pharm.	Study start: Dec. 2014	Little Rock, AR, USA
			Case-control	Age: child, adult, older adult		First posted: Dec. 3 2014	Centennial, CO, USA
			Prospective			Last update posted: Aug. 17 2017	Lakewood, CO, USA
			Outcome measures:				And 52 more
			Patients-reported outcomes	Sex: M and F			
NCT02751801	Health Burden of HPP	Recruiting	Observational	Enrollment: 17	Sheffield Teaching Hospitals NHS Foundation Trust	Study start: Nov. 2016	Academic Unit of Bone Metabolism, Sheffield, South Yorks, UK
			Case-only	Age: up to 100 years, child, adult, older adult		first posted: Apr. 26 2016	
			Retrospective				
			Outcome measures:	Sex: M and F		Last update posted: Nov. 24 2017	
			Description of healthcare use				
			Healthcare use appointments				
			Healthcare cost				

From ClinicalTrials.gov, as of August 23, 2018

HPP hypophosphatasia, *Alexion Pharm* Alexion Pharmaceuticals, *AEs* adverse events, *NA* not available, *SC* subcutaneous, *RGI-C scale* qualitative radiographic global impression of change, *PPi* inorganic pyrophosphate, *PLP* pyridoxal-5-phosphate, *PD* pharmacodynamics, *PK* pharmacokinetics

10.3.1 Preclinical Studies

In the first published preclinical study, TNSALP-null *Akp2*$^{-/-}$ mice (i.e. mice deprived of the murine TNSALP gene), a good animal model for infantile HPP (Fedde et al. 1999) were treated with daily subcutaneous injections of ENB-0040 at different doses (1, 2, or 8.2 mg/kg/day) for up to 52 days after birth. Seventy-five percent of those receiving the highest doses survived and showed normal physical activity, normal growth, no skeletal or dental alterations, and no epilepsy, while the median survival of untreated mice was only 18.5 days. Plasma levels of calcium, PPi and pyridoxal remained within normal range (Millán et al. 2008).

Another study on the same animal model investigated the dose-response relationship with different doses (0.5, 2.0, or 8.2 mg/kg/day) of subcutaneous ENB-0040 for 43 days after birth. Bone defects were prevented in 80% of mice with doses of 2.8–3.2 mg/kg/day, depending on the skeletal site. Long bones apparently responded positively to lower doses. Overall, bone mineralization, bone length, body weight and median survival improved with increasing doses. Interestingly, the authors observed that urinary PPi (a good marker for the diagnosis of HPP) is probably not useful in the follow-up of ERT, since it remained elevated in all treated groups (Yadav et al. 2011). Further studies on *Akp2*$^{-/-}$ mice reported that ENB-0040 can prevent hypomineralization of alveolar bone, dentin, and cementum, as well as enamel defects (McKee et al. 2011; Foster et al. 2013).

10.3.2 Studies in Infants and Children

Following these preliminary positive results in animal models, ENB-0040 was investigated in infants and children with very severe forms of HPP.

In 2012, Rodriguez et al. used ENB-0040 in a preterm 3-week-old infant with HPP, with severe respiratory insufficiency requiring mechanical ventilation since birth. After 12 weeks of treatment, lung function and chest wall mechanics were improved with respect to a baseline evaluation at age 8 weeks (after 5 weeks of treatment), and oxygen flux could be reduced. At age 32 weeks, the infant could be discharged from the hospital, but unfortunately, only 2 weeks later, had to be rehospitalized because of fever, hypoxemia, pneumonia and shock, and in a few days he died from septic shock with multi-organ failure, disseminated intravascular coagulopathy, hypoxemic respiratory failure and acute respiratory distress syndrome (Rodriguez et al. 2012).

In the same year, the first results of a Phase 2 multinational open-label study of ERT treatment with ENB-0040 in infants and young children with life-threatening HPP (ENB-002-08; ClinicalTrials.gov number: NCT00744042) were published. The study enrolled 11 patients (7 girls and 4 boys, aged 2 weeks to 3 years; 5 with perinatal HPP and 6 with infantile HPP). All children presented typical HPP symptoms (failure to thrive, severe hypomineralization, fractures, substantial motor delay or regression) within the first 6 months of life. Those with erupted teeth had lost dentition, and all but two had nephrocalcinosis or renal stones. Ten of the 11 patients required some respiratory support (5 at baseline and 5 at a later time) during the study. They were treated with a single intravenous infusion (2 mg/kg) of ENB-0040 initially, followed by subcutaneous injections three times a week (1 mg/kg, to be increased up to 3 mg/kg in case of worsening pulmonary function, failure to thrive or absence of skeletal improvement). One child was withdrawn after the initial intravenous dose, because of disturbing symptoms. Ten children completed 6 months of treatment and nine completed 1 year. One of them died from sepsis (unrelated to treatment) during the 7th month of treatment. Skeletal healing became apparent within the first 24 weeks of treatment in all patients but one, with increased BMC (bone mineral content), improved endochondral and membranous bone formation, fracture healing, reduced deformity, resolution of radiolucencies and sclerosis, improved bone modeling and remodeling. One patient who had

no visible bone at baseline did not have an early response to treatment, but showed some remineralization at 48 weeks. After 6 months of therapy, nine patients showed healing of rickets, as well as improvement in pulmonary function and developmental milestones. Serum PTH had an increasing trend in parallel with bone mineralization, allowing liberalization of dietary calcium intake. Serum calcium and phosphate levels showed only transient minor fluctuations. Plasma levels of TNSALP substrates (PPi and PLP) decreased; in particular, PPi (measured in five patients) significantly decreased over the 48 weeks of treatment. Apparently, ERT had no effect on craniosynostosis and two patients required cranial surgery. The most common ERT-related adverse event was mild, transient erythema localized at the injection site. Nephrocalcinosis did not progress after the first 6 months of treatment and even improved in some patients. No ectopic calcifications were observed. The increased bone mineralization process did not lead to hypocalcemia ("hungry bones"). There were no signs of drug hypersensitivity or tachyphylaxis, and only four patients developed low titers of anti-ENB-0040 antibodies, with no evident clinical, biochemical, or autoimmune abnormalities at 48 weeks of treatment. Three serious adverse events in three patients (one each of respiratory distress, craniosynostosis, conductive hearing loss), however, were considered as possibly related to the treatment (Whyte et al. 2012a).

In 2014, the results of an extension (NCT01205152) of the previous study were presented at a medical conference in Canada. The nine patients who had completed the 1st year of treatment, continued for two more years (until month 36). Asfotase alfa (the ENB-0040 was given the new name in the meantime) was well tolerated, and the improvement in skeletal mineralization continued. Only one patient continued to need supplemental oxygen at the last assessment after 3 years. Survival at 3 years was 90%, while in previous studies in comparable populations it was less than 50% (Whyte et al. 2014). Still later, Whyte communicated that after more than 5 years of treatment, these patients were showing further improvements and none was requiring respiratory support (Whyte 2017a), and that similarly positive results were being observed in an ongoing study on a larger number of comparable patients (Liese et al. 2016).

At another 2014 medical conference, Rockman-Greenberg et al. presented the results of an ongoing Phase II, open-label, global, multicenter trial (NCT01176266) on 15 children with HPP (age range 0.1–304 weeks; N = 8 age <22 weeks; N = 7 age 168–304 weeks). The patients received asfotase alfa as a subcutaneous injection (initial dose 2 mg/kg, three times per week; with adjustments permitted). The 13 patients with at least 6 months of follow-up data were evaluated. Significantly improved bone mineralization was observed at week 12 in 6/14 patients (42.9%), at week 24 in 8/12 patients (66.7%), at week 36 in 10/11 patients (90.9%) and at week 48 in 10/10 patients (100%). Eight of the 15 patients required some respiratory support during the study (5 at baseline and 3 at a later time); 4 of them still needed respiratory support at the last assessment. The overall survival was 93% (one patient withdrew on day 16 and died on day 22, from disease-related complications, not attributed to the drug). There were no serious drug-related adverse events, the most common events being mild to moderate reactions at injection site (Rockman-Greenberg et al. 2014).

After 2014, the results of further Phase 2 clinical trials of asfotase alfa in infants and adolescents with perinatal, infantile, or childhood HPP were presented at medical conferences and published in medical journals, confirming the positive effects of ERT on bone mineralization, growth, respiratory function, and mobility.

The first of these trials (NCT00952484, extended by NCT01203826) evaluated the efficacy and safety of asfotase alfa treatment in children aged 6–12 years, with rickets and impaired physical function due to HPP. Thirteen children were initially enrolled, one withdrew, 12 continued treatment for up to 5 years. Significant improvement in bone mineralization was observed after 6 months of treatment, then further improvements occurred and persisted after 5 years. In particular, 9/13 patients (69%) could be classified as "responders" (i.e. obtaining a

Radiographic Global Impression of Change (RGI-C) score ≥2) after 6 months of therapy, compared to 1/16 (6%) in the control group. The percentage of treated patients classified as "responders" increased to 75% after 2 years of treatment, 88% after 3 years, and 92% after 5 years. Overall, growth, strength, motor function, agility, and quality of life improved, in many cases achieving normal values for age- and sex-matched peers, and were sustained at 5 years. Pain and disability resolved in most cases. The most common adverse effects were mild to moderate reactions at injection site. No clinically important ectopic calcification or treatment resistance were observed. Low titers of anti–asfotase alfa antibody appeared in all patients. These results confirmed that ERT with asfotase alfa has good and sustained efficacy and a good safety profile for children suffering from HPP (Fig. 10.6) (Whyte et al. 2016a).

In another article (Whyte et al. 2016b), the survival rates observed in two ongoing, multicenter, open-label, phase 2 interventional studies of asfotase alfa treatment in neonates and infants with severe HPP (NCT00744042/NCT01205152 and NCT01176266) were compared with those observed in a retrospective, multinational, non-interventional, natural history study (NCT01419028) of patients with perinatal or infantile HPP with rachitic chest deformity, respiratory compromise, or vitamin B6–dependent seizures, used as controls. Thirty-seven patients were included in the two ERT studies (median treatment duration, 2.7 years) and 48 (with similar age and HPP severity) in the historical control study. The main outcome measures were survival, skeletal health (radiographically evaluated), and ventilatory status. Ninety-five percent (35/37) of treated patients were alive at 1 year of age versus 42% (20/48) of the historical controls, 84% (31/37) versus 27% (13/48) at 5 years of age. The median survival for the historical controls was 8.9 months, with respiratory complications or failure as the primary cause of death. The better respiratory outcome was accompanied by radiographically demonstrated improvements in skeletal mineralization. In particular, only 5% (1/20) of the historical controls who required respiratory assistance survived, while 76% (16/21) of the ventilated treated patients survived, and 75% of them (12/16) could be weaned from respiratory support. These results confirmed that asfotase alfa significantly improves skeletal mineralization, respiratory

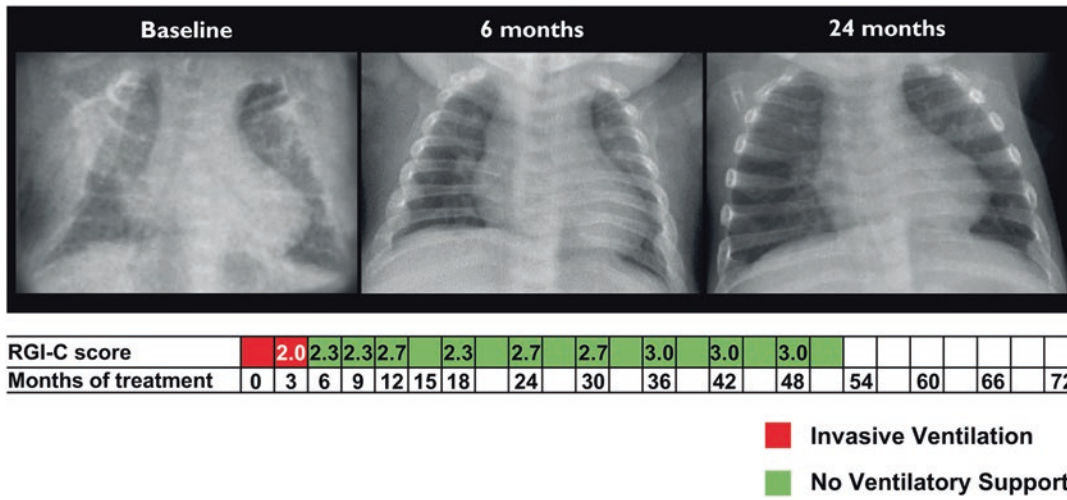

Fig. 10.6 Radiographic changes with asfotase alfa treatment in a patient with perinatal/infantile HPP. Better rib mineralization, chest structure, and thoracic volume with improved ventilatory status in an infant with hypophosphatasia (5.1 weeks of age at treatment baseline). RGI-C, Radiographic Global Impression of Change scale. (From Whyte et al. 2016b, reproduced with permission)

function and prolong survival in life-threatening perinatal and infantile HPP.

Another 1-year open-label, multicentre, prospective trial (NCT02456038) included 13 patients (9 females, 4 males; age 0 days-34 years) with *ALPL* gene mutations: 6 patients had perinatal HPP, 5 infantile HPP, 1 childhood HPP, 1 adult HPP. All but one were treated with asfotase alfa (2 mg/kg three times weekly). The primary outcome measure was safety, determined on the basis of observed adverse events (AEs), and the secondary outcome measure was efficacy, determined on the basis of overall survival, respiratory status, rickets severity and gross motor development. The more severe AEs (convulsion and hypocalcaemia, possibly related to treatment) were observed in one patient with perinatal HPP. Three patients with infantile HPP had hypercalcemia and/or hyperphosphatemia, requiring low-calcium and/or low-phosphate formula. The most frequent AEs were injection site reactions. Regarding efficacy, all patients survived, with improvements in radiographic findings, respiratory function, and development (Kitaoka et al. 2017).

To complete this survey, a few interesting case reports on ERT in perinatal or infantile HPP deserve mention.

Okazaki et al. (2016) report a very interesting case of a female infant with perinatal lethal HPP diagnosed in utero. At birth, she had severe skeletal hypomineralization and severe respiratory insufficiency requiring invasive ventilation and deep sedation. She was treated with asfotase alfa, starting the day after birth, and improvements in bone mineralization were visible at 3 weeks of age. She required calcium supplementation for the first 3 months of treatment, to correct serious hypocalcemia with convulsions. At the time of report, the baby was no more requiring mechanical ventilation and had survived for over 1 year.

In another case report, a dramatic clinical improvement was observed in a girl with infantile HPP, treated with asfotase alfa for more than 5 years (Fig. 10.7) (Simm and Savarirayan 2017).

A similar case was very recently reported by Oyachi et al. (2018). A newborn girl presented with respiratory insufficiency and seizures. Low ALP activity and high pyridoxal phosphate levels were measured in the umbilical cord blood, and severe rickets-like bone alterations were observed radiologically. She was treated with mechanical ventilation and pyridoxine hydrochloride. Perinatal severe HPP was diagnosed, and asfotase alfa treatment was started at 6 days of age. Genetic analysis revealed compound heterozygous mutations of the *ALPL* gene (c.1559delT/p.Ser188Pro). With ERT, skeletal mineralization and respiratory insufficiency improved. Seizures ceased and pyridoxine hydrochloride was tapered off at age 1 year. There were no remarkable side-effects.

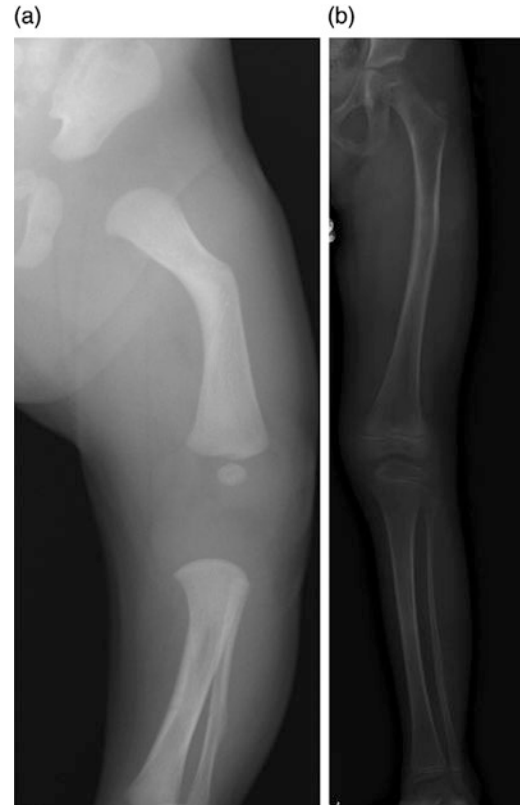

Fig. 10.7 Comparison of left femur, from (**a**) 2 months of age, before use of enzyme replacement therapy and (**b**) at age 5 years 10 months during asfotase alfa, demonstrating significant reduction in femoral bowing. (From Simm and Savarirayan 2017, reproduced with permission)

Finally, on the negative side, Costain et al. (2017) describe in great detail the case of a female baby with perinatal HPP for whom ERT with asfotase alfa was not successful. The baby

was born with signs of poor bone mineralization and severe respiratory insufficiency, that required ventilation by continuous positive airway pressure (CPAP). On day 13, after a diagnosis of perinatal HPP, she was put on ERT with asfotase alfa. In the following months, notwithstanding some radiological improvement in bone mineralization, her chest wall and lungs failed to grow, she could never be weaned from mechanical ventilatory assistance and her general condition remained very poor. Eventually, the multidisciplinary healthcare team and the parents, after a difficult evaluation of the potential benefits versus the ongoing harms, made a decision to stop ERT, extubate, and provide only supportive care. On day 100, the baby died.

10.3.3 Studies in Adolescents and Adults

Regarding the less severe forms of HPP, there are only very few studies and the benefits of treatment with asfotase alfa have not yet been demonstrated in mild childhood HPP, adult HPP, and odontoHPP.

A poster (Kishnani et al. 2012) and a short communication (Whyte et al. 2012b) is all that we could find about an open-label, multicenter, randomized, controlled Phase II study of the safety and efficacy of asfotase alfa in 6 adolescents and 13 adults with HPP (mean age 42 years, range 14–68 years) (NCT01163149). Thirteen subjects received daily subcutaneous injection of asfotase alfa (dose 2.1 (N = 7) or 3.5 (N = 6) mg/kg/week) and six received no treatment (controls). After 24 weeks of treatment with asfotase alfa, TNSALP substrate levels (serum PPi and serum PLP) were significantly decreased, motor function (6-min walk test) was also improved, although by only 26 m on average, and treatment was well tolerated, with no serious adverse events related to treatment. Hofmann et al. (2016) cited an extension of this study in which all these subjects (including the non-treated controls) were treated with 3.5 mg/kg/week of asfotase alfa for another 24 weeks, then with 1 mg/kg/day 6 days/week for an additional 48 weeks (or until regulatory approval of the drug), but no data on this extension have apparently been published.

There are very few other data on adults. Remde et al. (2017) described a hemodialyzed 59-year old woman with childhood-onset HPP and a history of multiple fractures and orthopedic procedures that after 13 months of treatment with asfotase alfa had a dramatic increase in quality of life, increased mobility, reduction in pain drugs, and significant improvement in bone mineralization, with consolidation of existing fractures and no new fractures.

At the time of writing, asfotase alfa has not been approved for use in adult HPP, although some possible benefits could be expected, at least in selected cases, on the basis of the experience in children and the few data available for older patients.

Shapiro and Lewiecki (2017) discuss the problems and perspectives of ERT with asfotase alfa in adults, and present some suggestions about the possible criteria for treatment. The extent of HPP-related disability should always be a major criterion. Most important is a history of childhood involvement (before age 18 years), such as early loss of primary or secondary teeth, craniosynostosis, gait disturbance, developmental delays, skeletal deformity, bone pain or fractures. In the absence of childhood symptoms, these other criteria are suggested: musculoskeletal pain requiring prescription pain medications; disabling polyarthropathy and/or chondrocalcinosis; major low-trauma fractures (e.g., spine, hip, humerus, pelvis) attributable to HPP; delayed or incomplete fracture healing or fracture nonunion, especially if requiring orthopedic surgery; disabling functional impairment (e.g., mobility, gait, activities of daily living) assessed by validated measures; low bone mineral density (BMD) (T-score ≤ -2.5 in postmenopausal women and men aged ≥ 50 years; or Z-score ≤ -2.0 in younger adults) by dual-energy X-ray absorptiometry (DXA); radiological evidence of nephrocalcinosis.

The benefits and risks of asfotase alfa ERT in adults are still undetermined, as well as the optimal dose, need for dose adjustments, and duration of therapy. Adverse effects of long-term ERT

are still unknown. The currently high cost of therapy is obviously an important aspect and might justify the use of asfotase alfa only in severe cases of HPP.

10.3.4 Adverse Reactions to Asfotase Alfa

The adverse reactions to asfotase alfa (Strensiq®) reported in clinical trials (on a total of 79 patients with perinatal or infantile-onset HPP, and 20 patients with juvenile-onset HPP) are presented in detail in the Strensiq® prescribing information document (Strensiq® Alexion Prescription Information 2018) and are discussed in some review articles (Hofmann et al. 2016; Kishnani et al. 2017).

The most common reactions were injection site reactions (in 63% of patients). Other common adverse reactions included lipodystrophy (28%), ectopic calcifications (14%), and hypersensitivity reactions (12%). Injection site reactions, lipodystrophy and ectopic calcification were higher in patients with juvenile-onset HPP than in patients with perinatal/infantile-onset. Most injection-site reactions and injection-associated reactions were nonserious, mild to moderate, and resolved within a week. They were more common in patients receiving six injections per week compared with those receiving only three injections per week. Localized lipodystrophy (atrophy or hypertrophy) was observed after several months of injections. Rotation of injection site seems to reduce the risk of site reactions. Ectopic calcifications were observed in eyes (including cornea and conjunctiva) and kidneys (nephrocalcinosis), without evidence of visual disturbances or changes in renal function. There was insufficient information to determine whether these manifestations were disease- or drug-related.

Hypersensitivity reactions (12% of patients) included vomiting, erythema, fever, irritability, nausea, pain, rigor/chills, oral hypoesthesia, headache, flushing, and anaphylaxis (1%). Less common adverse reactions (reported in <1%) were hypocalcemia, renal stones, chronic hepatitis, decreased vitamin B6.

Since increased levels of parathyroid hormone (PTH) and decreased levels of calcium have been reported during treatment with asfotase alfa, particularly in the first 12 weeks, PTH and calcium levels should be monitored during treatment and supplementation with calcium and/or vitamin D may be indicated (Hofmann et al. 2016).

10.3.5 Immunogenicity

Being a protein, asfotase alfa has a potential for immunogenicity. In clinical trials, antibodies to asfotase alfa have been found in 76 (78%) out of 98 patients tested. Of these, 34 (45%) had neutralizing antibodies. However, the development of antibodies has not been associated with clinical resistance to the drug (Hofmann et al. 2016; Whyte 2017a).

10.3.6 Interactions

There are no data on the interactions of asfotase alfa with other therapeutic drugs (Hofmann et al. 2016).

10.4 The Goals of Therapy with Asfotase Alfa and Its Monitoring

The treatment of HPP patients with asfotase alfa poses different problems depending on the disease subtype and severity as well as on the patient's history and clinical manifestations. In younger patients, the main treatment goals are improved skeletal mineralization, prevention and/or control of complications (seizures, respiratory insufficiency, renal failure, neurosurgical problems), reduction of pain, improved growth and development, improved mobility. In particular, in perinatal or infantile HPP, where survival is the primary goal, respiratory function and seizure control are urgent problems, and ventilatory support is often required. In childhood HPP, depending on disease severity, longer-term goals, such as growth, mobility, and bone mineralization, can

be pursued. In adults, HPP-related fractures are the most important problem, and treatment should aim at improving fracture healing and reducing the risk of new fractures. Other treatment goals are functional improvement (strength, endurance, fatigue, pain). Oral health is another important treatment goal for patients of all ages, and overall quality of life should of course be the guiding light of the caregivers.

In the absence of published guidelines, the monitoring of asfotase alfa therapy in the different forms of HPP and the different age groups was discussed by an international panel of experienced physicians, convened in 2016 by the producer of asfotase alfa, Alexion Pharmaceuticals Inc. The conclusions and recommendations of this important meeting (regarding the type and frequency of clinical and radiological evaluations, laboratory tests, and assessments of efficacy/safety to be performed during the course of treatment) are presented in a consensus report by Kishnani et al. (2017).

10.5 Challenges and Future Perspectives

Asfotase alfa treatment for HPP is now widely recognized, and hopefully, the accumulation of experience with it will not only increase the benefits for patients, but will also help us develop a better understanding of the pathophysiology of HPP and its extremely variable picture and severity. There are, however, many aspects that require further investigations, and as happens with all new treatments, problems and challenges have emerged.

10.5.1 Problems and Challenges

Treatment with asfotase alfa is a delicate matter, and before starting it, a correct diagnosis of HPP must be made (for diagnostic challenges, see above Sect. 10.1.6). The inappropriate use of this enzyme in conditions other than HPP could result in serious adverse consequences from excessive mineralization.

When asfotase alfa therapy is indicated, as in perinatal, infantile, and severe childhood HPP, a timely initiation is important to minimize the risks from respiratory and other complications and the need for intensive care. Perhaps in utero therapy of perinatal HPP will be possible in the future (Whyte 2017a).

Monitoring and follow-up are important. The dose of asfotase alfa, which is strictly based on the patient's weight, must be regularly adjusted because, should it become insufficient, clinical and radiological worsening may soon recur (Whyte 2017a).

Ectopic calcifications are a theoretically possible adverse effect of treatment with asfotase alfa. Should future clinical experience demonstrate the appropriateness of long-term or even life-long treatment regimens, the risk of vascular or cardiac valve calcifications will have to be considered as the patients' age increases, and deserves specific investigations (Hofmann et al. 2016; Whyte 2017a). There is also some evidence that high alkaline phosphatase activity could be associated with degenerative diseases of the central nervous system, and this risk must also be taken into account (Hofmann et al. 2016).

Throughout life, the physiological production of ALP varies according to skeletal growth and development, bone modeling and remodeling activity, and fracture repair: whether dose adaptation according to the physiological needs is appropriate has to be determined by further studies. Also, the possibility of intermittent treatment regimens in milder forms of HPP, which might require ERT only in challenge situations like fractures or implant surgery, should be investigated (Hofmann et al. 2016).

10.5.2 Future Perspectives

Currently, asfotase alfa treatment requires three or six administrations per week, with up to two subcutaneous injections per administration if high doses are needed. The half-life of asfotase alfa following subcutaneous administration is 5 days (Strensiq® Alexion Prescription Information 2018). Some future drug modifica-

tion might allow to increase the interval between injections (Orimo 2016).

Bone marrow stem-cell transplantation has been attempted in the past, with little success (Whyte et al. 2003; Taketani et al. 2015), but the efficacy and safety of combination treatment of asfotase alfa and stem-cell transplantation may deserve evaluation (Orimo 2016).

Finally, ex-vivo gene therapy using bone marrow stem cells is also being evaluated, and has already been successfully attempted in TNSALP-null *Akp2*$^{-/-}$ mice (Yamamoto et al. 2011; Matsumoto et al. 2011). Use of a viral vector containing the *ALPL* gene is also being explored, and an experiment with TNSALP-null *Akp2*$^{-/-}$ mice has already been made with positive results (Iijima et al. 2015). Of course, this is only a very preliminary step, because the safety of vector administration must be demonstrated before its use in humans, and the vector with the highest transduction efficacy must be identified (Orimo 2016).

10.6 Conclusions

HPP is a very complex, multi-systemic disease, with a very variable, multi-faceted presentation and a wide range of severity, from very mild to lethal. In its different forms, HPP can reveal itself at different ages, and may involve different organs (bone, tooth, muscle, lung, kidney, gastrointestinal tract, peripheral and central nervous system). Its pathophysiology is still not completely understood in all its aspects and the diagnosis can be difficult and is often delayed.

Once a diagnosis of HPP is established, ERT with asfotase alfa is the only effective and promising treatment currently available, especially for the severe forms of infancy and childhood. The first clinical trials with asfotase alfa have demonstrated clinical, radiographic, and biochemical improvements in infants and children with perinatal, infantile or childhood HPP, persisting after over 5 years of treatment in many cases.

Notwithstanding the inconvenience of frequent injections (with the related adverse reactions), this therapy has demonstrated a good safety profile, but its long-term efficacy and safety are unknown. The treatment must be carefully tailored to individual requirements and its management and follow-up are highly demanding tasks, requiring the involvement of an experienced and dedicated multidisciplinary team. Finally, the high cost of therapy is a major issue, that cannot be addressed here.

Some positive results with asfotase alfa have also been reported in adolescents and adults with milder forms of HPP, but the benefits of asfotase alfa have not been definitely demonstrated in these cases and alternative treatments could eventually prove preferable.

As a last note, considering the rarity of the disease, the development of medical centers with the required multidisciplinary expertise is a major issue, and underlines the necessity for a regular, timely sharing of clinical observations and experiences via national and international "rare diseases" networks, in order to build a sound evidence base on the natural history of HPP and the long-term impact of treatment.

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