Full Length Article

Five-year efficacy and safety of asfotase alfa therapy for adults and adolescents with hypophosphatasia☆

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ABSTRACT

Hypophosphatasia (HPP) features low tissue-nonspecific alkaline phosphatase (TNSALP) isoenzyme activity resulting in extracellular accumulation of its substrates including pyridoxal 5′-phosphate (PLP), the principal circulating form of vitamin B6, and inorganic pyrophosphate (PPi), a potent inhibitor of mineralization. Asfotase alfa is an enzyme replacement therapy developed to treat HPP. This multinational, randomized, open-label study (NCT01163149; EudraCT 2010-019850-42) evaluated the efficacy and safety of asfotase alfa in adults and adolescents 13–66 years of age with HPP. The study comprised a 6-month primary treatment period and a 4.5-year extension phase. In the primary treatment period, 19 patients were randomized to receive asfotase alfa 0.3 mg/kg/d subcutaneously (SC; n = 7), asfotase alfa 0.5 mg/kg/d SC (n = 6), or no treatment (control; n = 6) for 6 months. In the extension phase, patients received asfotase alfa (0.5 mg/kg/d for 6 mo–1 y, then 1 mg/kg/d 6 d/wk). During the primary treatment period, changes from Baseline to Month 6 in plasma PLP and PPi concentrations (coprimary efficacy measure) were greater in the combined asfotase alfa group compared with the control group, reaching statistical significance for PLP (P = 0.0285) but not for PPi (P = 0.0715). However, for the total cohort, the within subject changes in both PLP and PPi after 6 months and over 5 years of treatment with asfotase alfa were significant (P < 0.05). Secondary efficacy measures included transiliac crest histomorphometry, dual-energy X-ray absorptiometry (DXA), and the 6-Minute Walk Test (6MWT). A significant decrease from Baseline in mineralization lag time was observed in the combined asfotase alfa group at Year 1. There were no significant differences between treated and control patients in DXA mean bone mineral density results at 6 months; Z-scores and T-scores were within the expected range for age at Baseline and remained so over 5 years of treatment. On the 6MWT, median (min, max) distance walked increased from 355 (10, 620; n = 19) meters before treatment to 450 (280, 707; n = 13) meters at 5 years (P < 0.05). Results for the exploratory outcome measures suggested improvements in gross motor function, muscle strength, and patient-

reported functional disability over 5 years of treatment. There were no deaths during this study. Asfotase alfa was generally well tolerated; the most common adverse events were mild to moderate injection site reactions. This study suggests that in adults and adolescents with pediatric-onset HPP, treatment with asfotase alfa is associated with normalization of circulating TNSALP substrate levels and improved functional abilities.

1. Introduction

Hypophosphatasia (HPP) is the rare metabolic disease caused by loss of function mutation(s) of the gene (*ALPL*) that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) [1–3]. Inheritance of HPP is autosomal recessive or dominant, with > 340 mutations identified to date [4,5]. Low TNSALP activity on cell surfaces in HPP leads to extracellular accumulation of the TNSALP substrates pyridoxal 5′-phosphate (PLP) [1,6,7], inorganic pyrophosphate (PPi) [1,8,9], and phosphoethanolamine [1,10]. PPi is an inhibitor of mineralization and its superabundance in HPP can cause rickets in children or osteomalacia in adults [1,10,11]. The signs, symptoms, and sequelae of HPP can present from in utero to adulthood and can vary widely among patients [11–13]. In the case of adolescents and adults, the onset of signs and symptoms of HPP may have occurred in infancy or later in childhood or adulthood [14,15]. HPP in adults commonly presents with recurring/poorly healing metatarsal fractures, pseudo-fractures, loss of teeth and sometimes muscle weakness, altered ambulation, and bone, joint, and muscle pain [14,16–22]. Some adults may manifest isolated signs or symptoms, such as tooth loss or arthropathy, without overt bone disease [23].

Asfotase alfa (Alexion Pharmaceuticals, Inc., Boston, MA, USA) is a human recombinant enzyme replacement therapy developed to treat HPP [24]. Studies of affected infants and children showed that therapy with asfotase alfa improved skeletal mineralization and other abnormalities, growth, mobility, respiratory function, survival, and pain, with benefits sustained up to 7 years of therapy [25–28]. The objective of the current study was to assess the efficacy and safety of asfotase alfa in adults and adolescents with HPP.

2. Methods

2.1. Study design

This study complied with the World Medical Association Declaration of Helsinki and the International Conference on Harmonisation E6 Guideline for Good Clinical Practice. Approval was obtained from the institutional review board or research ethics board at each investigative site. All patients or their legal guardians provided informed consent or assent.

This multicenter, randomized, open-label, Phase 2 study (NCT01163149) was conducted at 3 sites (2 in the United States and 1 in Canada). It consisted of a 6-month primary treatment period followed by an open-label extension phase. In the primary treatment period, patients were randomized to 1 of 3 groups: asfotase alfa administered subcutaneously (SC) at 0.3 mg/kg/d (2.1 mg/kg/wk) or 0.5 mg/kg/d (3.5 mg/kg/wk) or no treatment (control group) (Fig. 1). During the extension phase all patients initially received asfotase alfa 0.5 mg/kg/d, but after approximately 6 months to 1 year the dose was increased to 1 mg/kg/d for 6 d/wk (6 mg/kg/wk) under a protocol amendment; for 1 patient this was not implemented. The change to 6 mg/kg/wk was made to match the lowest dose showing efficacy in children with HPP [27].

2.2. Inclusion and exclusion criteria

Eligibility criteria were age 13–65 years and a pre-established diagnosis of HPP based on medical history and findings consistent with HPP, including subnormal age-adjusted serum alkaline phosphatase (ALP) activity; high plasma PLP concentration ($\geq 2\times$ upper limit of normal; no vitamin B_6 administered ≥ 1 week before specimen collection); and osteopenia on skeletal radiographs. Patients must have had osteomalacia documented by iliac crest biopsy per protocol to participate in the study. (Additional details are provided in the Supplemental materials.)

Principal exclusion criteria were subnormal serum calcium or phosphate concentrations or 25-hydroxyvitamin D concentrations < 20 ng/mL (patients failing initial screening because of low 25-hydroxyvitamin D could be rescreened at the discretion of the investigator after vitamin D supplementation); serum creatinine or parathyroid hormone (PTH) concentrations above the upper limit of normal; off-label treatment with a PTH analog within 6 months; or use of bisphosphonates within 2 years of study entry or for > 2 years at any time. Patients with prior bisphosphonate use were included only if they had normal or elevated levels of serum C-telopeptide and urine N-telopeptide or deoxypyridinoline.

2.3. Efficacy assessments

2.3.1. Coprimary efficacy measure

The coprimary efficacy measure was change in plasma PLP (ng/mL) and PPi (μM) concentrations from Baseline to Month 6 in the combined
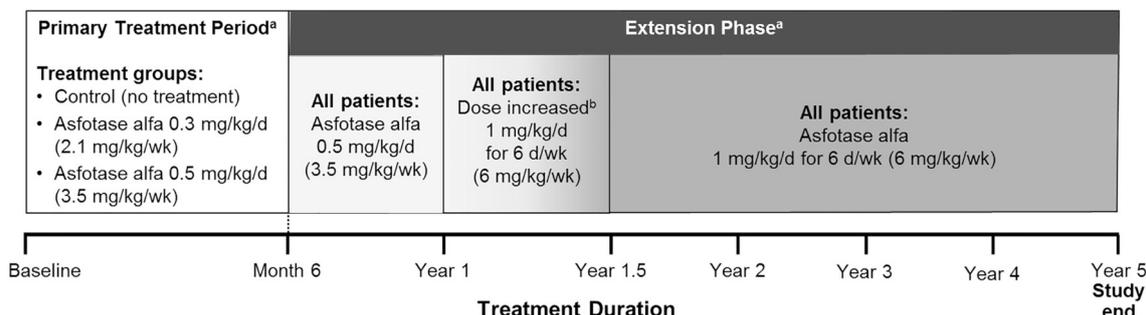
Fig. 1. Study design.
[a]Asfotase alfa dose was adjusted for patient body weight every 3 months; maximum daily dose was 80 mg unless the investigator, after consultation with the medical monitor, approved a higher dose.
[b]Dose increased by protocol amendment in all patients. During the extension phase, all patients initially received daily doses of asfotase alfa 0.5 mg/kg/d for approximately 6 months to 1 year, then increased to 1 mg/kg 6 d/wk.

asfotase alfa treatment group that received either dose of asfotase alfa (0.3 or 0.5 mg/kg/d) versus the control group (see Supplemental materials). Vacutainer tubes for this blood sampling contained levamisole to inhibit the high ALP activity from asfotase alfa.

2.3.2. Secondary efficacy measures

2.3.2.1. Bone mineralization and bone mineral density. Transiliac crest biopsy was performed at Baseline and Year 1 in the asfotase alfa group and at Baseline and Month 6 in the control group. Instructions for preparing and handling the biopsy sample were provided to each site. Three patients (1 each in the control group, 0.3 mg/kg/d group, and 0.5 mg/kg/d group) who had undergone pretreatment bone biopsy in a prior Phase 1, dose-escalating, pharmacokinetic study of asfotase alfa (ENB-001-08/NCT00739505) had their biopsy results used as Baseline values for the current study. The current Phase 2 study began enrolling patients approximately 2 years after the 2-month Phase 1 study. To label the bone for dynamic histomorphometric assessment, patients received tetracycline for 2 3-day periods separated by approximately 2 weeks; biopsies were performed approximately 21 days after the second tetracycline dosing period. To ensure consistency across the asfotase alfa development program, all bone biopsy samples to assess the histomorphometric changes during the study were also analyzed at Shriners Hospital for Children, Montreal (Quebec, Canada). Percent of healthy mean values for bone biopsy parameters were calculated as observed value/healthy mean × 100, based on reference values available for males and females 1.5–22.9 years of age [29] and for postmenopausal women 45–74 years of age [30]. A score of 100% would indicate no difference from the healthy population.

Dual-energy X-ray absorptiometry (DXA) was performed at Baseline and every 6 months thereafter. Bone mineral density (BMD) Z-scores were calculated for the lumbar spine, femoral neck (or total hip), and whole body. A Z-score of −2.0 or lower is considered 'below the expected range for age' [31]. T-scores were calculated for patients older than 50 years of age. Postmenopausal status was not systematically captured in the study. The type of DXA machine and software did not change for each patient. A phantom was not used to calibrate the results across the study sites.

2.3.2.2. Walking ability. Walking ability was measured using the 6-Minute Walk Test (6MWT) performed in accordance with American Thoracic Society guidelines (2002) [32] by a licensed physical therapist at Screening, Baseline, Month 3, Month 6, and every 6 months thereafter (also performed at Month 9 [3 months after initiating asfotase alfa] in the control group). When possible, the 6MWT was performed before an injection of asfotase alfa and bone biopsy. The primary measurement for the 6MWT was distance walked (in meters) for all patients who attempted the 6MWT (i.e., walking the full 6 minutes was not required). The % predicted value for the 6MWT, defined as the % of normal predicted distance walked based on age, sex, and height [33,34], was calculated if the patient walked the full 6 minutes and was ≤65 years of age. Use of ambulatory assistive devices during the 6MWT was not collected systematically, and was not an efficacy measure of the study, but was sometimes documented in the investigator notes during assessment. If a patient was wheelchair bound, such that they could not participate in the 6MWT, their use of assistive devices was not collected in this setting. The minimally clinically important difference (MCID) in 6MWT distance walked for adults and adolescents with pediatric-onset HPP is 31 m and 43 m, respectively [35].

2.3.3. Exploratory efficacy measures

The Bruininks-Oseretsky Test of Motor Proficiency, second edition (BOT-2), hand-held dynamometry (HHD), Lower Extremity Functional Scale (LEFS), and Brief Pain Inventory–Short Form (BPI-SF) were administered by a licensed physical therapist at Screening, Baseline, Month 3, Month 6, and every 6 months thereafter (also at Month 9

[3 months after initiating asfotase alfa] in the control group). When applicable, these assessments were performed before an asfotase alfa injection and bone biopsy.

2.3.3.1. Motor function. Gross motor function was evaluated using the Running Speed and Agility and the Strength subtests of the modified version of the BOT-2, a validated assessment of motor ability in individuals 4–21 years of age [36]. Portions of the BOT-2 were videotaped to assess qualitative changes in gross motor function. Because study patients were 13–66 years of age (only 6 were 13–21 years of age at enrollment), the BOT-2 total scores, rather than scaled or age-standardized scores, were reported.

2.3.3.2. Muscle strength. Muscle strength was measured using HHD [37] of the hip extensors and hip abductors.

2.3.3.3. Patient-reported functional disability. Patient-reported functional disability was evaluated using the validated LEFS, which is scored 0–80, with higher scores indicating better functioning [38]. The LEFS assessed performance of transitional movements (e.g., getting out of bath, rolling over in bed, getting into or out of a car), locomotion (e.g., walking, running), climbing stairs, and squatting. The MCID in the LEFS score is 9 points for patients with musculoskeletal dysfunction of the lower extremities [38].

2.3.3.4. Patient-reported pain. Pain was evaluated using the modified version of the BPI-SF, a validated questionnaire comprising 4 items that assess pain severity (0 = no pain, 10 = worst pain you can imagine) [39,40]. The total pain severity score (0 = no pain to 40 = worst pain) was calculated from the 4 pain severity items.

2.4. Safety and tolerability

Safety assessments included monitoring of any adverse events (AEs) at each study visit, including injection site reactions (ISRs) and injection-associated reactions (IARs). ISRs were treatment-related AEs localized to the site of administration of asfotase alfa that occurred any time after initiation of asfotase alfa treatment, and IARs were systemic signs, symptoms, or findings (e.g., generalized urticaria or itching, hypotension, respiratory distress) that occurred within 3 hours after asfotase alfa administration. Both ISRs and IARs were assessed by the investigator as possibly, probably, or definitely related to the study drug. Additional safety assessments included physical examination findings, laboratory assessments, and anti–asfotase alfa antibody testing (PPD Laboratories, Richmond, VA). Serum ALP activity was measured using a standard CLIA (Chemical Laboratory Improvement Amendments) chemistry panel assay. All patients underwent renal ultrasound and a complete ophthalmic examination at Baseline and every 6 months thereafter to assess for ectopic calcification. Occurrences of fractures were not collected systematically but, if reported, were captured as an AE.

2.5. Statistical analyses

Efficacy analyses were performed on the full analysis set (FAS), which included all randomized patients. The safety population included all patients who received ≥1 dose of asfotase alfa. Group comparisons were made between the combined asfotase alfa group and the control group in the primary treatment period. Analyses of within subject changes in the total cohort after exposure to asfotase alfa were also carried out to 5 years of treatment. Patients in the asfotase alfa groups began treatment at the onset of the primary treatment period, whereas the control group began 6 months later at the onset of the extension phase. Because of the timing of study visits, time points were approximated, with 48 weeks defined as 1 year.

For the coprimary efficacy measures of change in plasma

concentrations of PLP and PPi from Baseline to Month 6, comparisons between the combined treatment group and the control group were made using an exact Wilcoxon rank-sum test for each parameter using a 2-sided alpha of 0.05. Results from this testing were used to determine if the treated patients had statistically significantly larger changes from Baseline than the control group. Since 2-sided testing was used, the statistical superiority of asfotase alfa treatment was established when *P* values were < 0.05 and the Hodges-Lehman-Sen estimate of between-group differences in the change from Baseline favored treatment vs. control. Missing values at Month 6 were imputed using last observation carried forward.

Statistical comparisons between the control group and the combined treatment group for bone biopsy results was not performed because of the difference in timing of the bone biopsies between the 2 groups (Month 6 and Year 1, respectively; see Discussion). However, the mean within patient change from Baseline and corresponding 95% confidence intervals (CIs) were calculated; changes were considered statistically significant (*P* < 0.05) if the 95% CIs did not include 0 (zero). For other secondary measures, including BMD Z-scores, 6MWT distance walked, and % predicted distance walked in the primary treatment period, statistical comparisons between the pooled asfotase alfa group and the control group were performed using the exact Wilcoxon rank-sum test and a 2-sided alpha of 0.05; no corrections to the alpha threshold were made for multiple comparisons. Analyses of plasma PPi, plasma PLP, BMD Z-scores and T-scores, 6MWT distance walked, and 6MWT % predicted distance walked were also performed using pooled data from the primary treatment period and the extension phase (when all patients received asfotase alfa). The mean change from Baseline and corresponding 95% confidence intervals (CIs) were calculated for each time point; changes were considered statistically significant (*P* < 0.05) if the 95% CIs did not include 0. Although these statistical comparisons were performed, the study was not powered to

show differences in these secondary measures. No statistical analyses were performed for the exploratory efficacy measures of motor function, muscle strength, disability, and pain.

3. Results

3.1. Patients

Study disposition of patients is summarized in Fig. 2. Of the 22 patients who were screened, 19 were randomized to asfotase alfa 0.3 mg/kg/d (n = 7), asfotase alfa 0.5 mg/kg/d (n = 6), or control (n = 6) in the primary treatment period. All patients then received asfotase alfa in the extension phase as planned. Five patients discontinued asfotase alfa treatment before study end. Three of the 5 patients withdrew consent (asfotase alfa 0.3 mg/kg/d group, age: 16 years; asfotase alfa 0.3 mg/kg/d group, age: 14 years; control group, age: 14 years); although AEs were not reported by the investigator as the cause of discontinuation, all 3 had ongoing mild or moderate ISRs consisting of injection site atrophy, lipohypertrophy, and/or skin discoloration. A fourth patient (control group, age: 26 years) was discontinued because of noncompliance, and a fifth patient (asfotase alfa 0.5 mg/kg/d group, age: 55 years) was discontinued after approximately 5 years of treatment because of 2 serious AEs (SAEs; injection site hypersensitivity and anaphylactoid reaction). Thus, a total of 16 patients received asfotase alfa for ≥4 years and 15 patients received asfotase alfa for ≥5 years.

Patient demographics and Baseline characteristics, including HPP disease-related history, are summarized in Table 1. The median age at enrollment was considerably lower in the control group (21.0 years; range: 13–58 years) than in the combined asfotase alfa group (55.0 years; range: 14–66 years). Although 1 patient 66 years of age was enrolled, which was considered a protocol deviation, this deviation was

Fig. 2. Patient disposition.
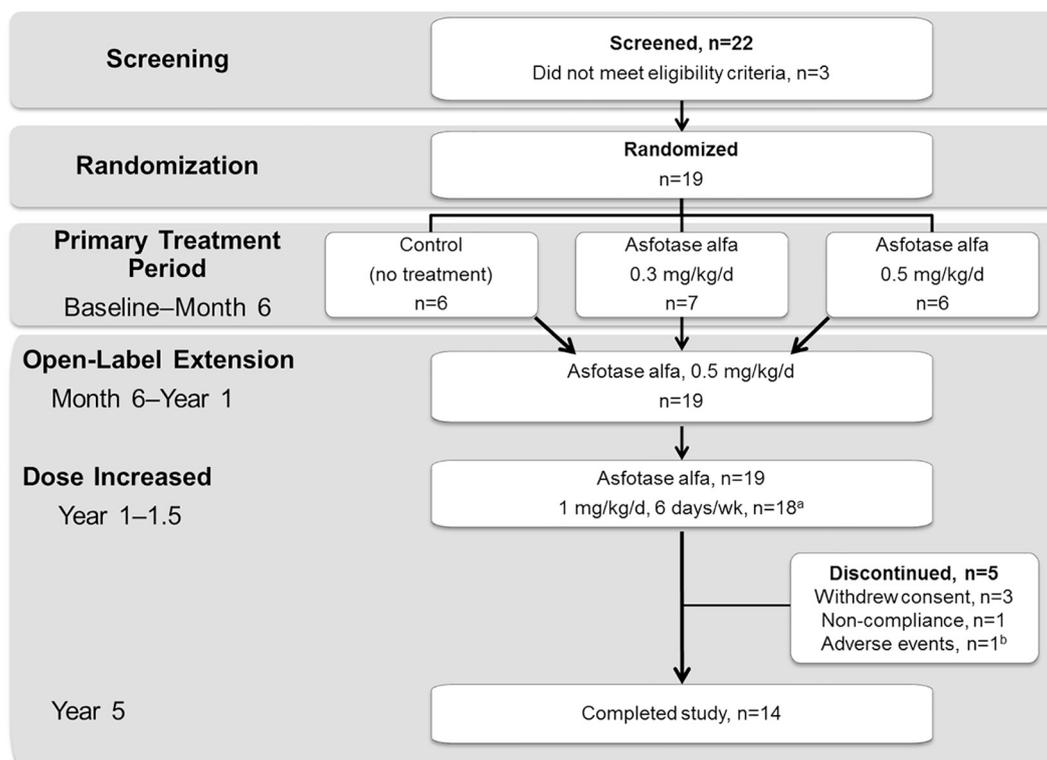[a]The dose of asfotase alfa was not increased to 1 mg/kg 6 d/wk in 1 patient; the dose was reduced to 0.5 mg/kg 3 d/wk at ~Year 1 because of multiple injection site reactions of atrophy, discoloration, and pruritus.
[b]One patient discontinued the study because of 2 serious adverse events of injection site hypersensitivity and anaphylactoid reaction after approximately 5 years of treatment.

Table 1
Baseline demographics and HPP-specific medical history.[a]

| | Total cohort[b] (N = 19) | Primary treatment period group assignment | |
		Control group (n = 6)	Treatment group[c] (n = 13)
Demographics			
Age at enrollment, y	**53 (13, 66)**	21 (13, 58)	55 (14, 66)
Adults (age ≥ 18 y), n (%)	**13 (68)**	3 (50)	10 (77)
Adolescents (age 13– < 18 y), n (%)	**6 (32)**	3 (50)	3 (23)
Age at HPP sign/symptom onset, y	**2.0 (0, 36)**	0.9 (0.2, 4)	2.0 (0, 36)
Adults (age ≥ 18 y)	**3.0 (0.1, 36)**	3.0 (0.8, 4)	2.5 (0.1, 36)
Adolescents (age 13– < 18 y)	**0.3 (0, 1)**	0.5 (0.2, 1)	0.2 (0, 0.5)
≥18 y, n (%)	**1 (5)**	0 (0)	1 (8)
< 18 y, n (%)	**18 (95)**	6 (100)	12 (92)
Female, n (%)	**12 (63)**	2 (33)	10 (77)
Race, white, n (%)	**18 (95)**	5 (83)	13 (100)
HPP-specific medical history			
Baseline serum ALP, U/L[d]	**18.0 (18, 45)**	23.5 (18, 45)	18.0 (18, 35)
Baseline plasma PLP, ng/mL[e]	**267.0 (29, 1590)**	237.0 (106, 906)	267.0 (29, 1590)
Baseline plasma PPi, μM[f]	**5.2 (2.2, 12.1)**	6.2 (4.2, 12.1)	5.1 (2.2, 8.2)
Patients with fractures, n (%)	**18 (95)**	6 (100)	12 (92)
Pseudofractures, n (%)	**12 (63)**	5 (83)	7 (54)
Nonhealing fractures, n (%)	**6 (32)**	1 (17)	5 (39)
Number of fractures	**6 (1, 30)**	5 (1, 8)	9.5 (1, 30)
Bone pain severity, n (%)			
Limits activity	**18 (95)**	5 (83)	13 (100)
Requires analgesics	**16 (84)**	5 (83)	11 (85)
Muscle complaints, n (%)			
Weakness	**17 (90)**	5 (83)	12 (92)
Pain	**14 (74)**	4 (67)	10 (77)
Joint complaints, n (%)			
Pain	**17 (90)**	5 (83)	12 (92)
Swelling	**7 (37)**	2 (33)	5 (39)
Unusual gait or walk/run, n (%)	**15 (79)**	4 (67)	11 (85)
Assistive devices (≥1) used at Screening, n (%)	**12 (63)**	4 (67)	8 (62)
Craniosynostosis, n (%)	**3 (16)**	0	3 (23)
Premature loss of deciduous teeth, n (%)	**16 (84)**	5 (83)	11 (85)
Loss of adult teeth, n (%)	**8 (42)**	1 (17)	7 (54)
Number of adult teeth remaining	**24 (0, 30)**	26 (0, 28)	23 (0, 30)
Hypercalcemia, n (%)	**6 (32)**	3 (50)	3 (23)
Hyperphosphatemia, n (%)	**6 (32)**	2 (33)	4 (31)
Gout, n (%)	**5 (26)**	2 (33)	3 (23)
Kidney stones,[g] n (%)	**4 (21)**	2 (33)	2 (15)

ALP = alkaline phosphatase; HPP = hypophosphatasia; PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

  [a] Values presented medians (min, max) unless noted otherwise.

  [b] Combined because all patients were treated in the extension phase.

  [c] Asfotase alfa groups combined for analysis.

  [d] Normal ALP ranges by age and sex per Covance, Inc.: *males* 10– < 15 years (95–385 U/L), 15– < 18 years (50–250 U/L), 18– < 50 years (31–129 UL), 50– < 60 years (35–131 U/L), and 60– < 70 years (35–125 U/L); *females* 10– < 15 years (51–300 U/L), 15– < 18 years (31–110 U/L), 18– < 50 years (31–106 U/L), and 50– < 70 years (35–123 U/L).

  [e] Normal PLP ranges by age category per Biotrial Bioanalytical Services: 5–18 years (5.7–61.2 ng/mL) and > 18 years (2.8–26.7 ng/mL).

  [f] Normal PPi ranges by age category per Charles River Laboratories: 13–18 years (< 0.8–4.8 μM) and > 18 years (1.0–5.8 μM).

  [g] As reported in the patient's medical history.

not considered likely to influence treatment effect, and therefore this patient was included in the analyses. Most patients were white (18/19 [94.7%]), female (12/19 [63.2%]), and adult (≥18 years of age; 13/19 [68.4%]). Ages reported for HPP sign or symptom onset ranged from 0 to 36 years (median: 2.0 years); most patients (14/19 [73.7%]) had childhood HPP, 4 (21.1%) had infantile HPP, and 1 (5.3%) had adult HPP. Median ALP activity at Baseline was 23.5 U/L for the control group and 18 U/L (the lower limit of detection) for the 0.3 and 0.5 mg groups [10,11]. All patients harbored *ALPL* mutations: 13 had compound heterozygous mutations, 5 had single copy dominant mutations, and 1 had a unique splice mutation [4]. The most common *ALPL* mutations were c.571G > A (8/19 patients [42.1%]), c.526G > A (4/19 [21.1%]), and c.1001G > A (4/19 [21.1%]).

3.2. Efficacy

3.2.1. Coprimary efficacy measure: PLP and PPi

Median changes from Baseline to Month 6 in plasma concentrations of PLP (Fig. 3a) and PPi (Fig. 3b) were greater in the combined asfotase alfa group (n = 13) compared with the control group (n = 6). The difference between groups was statistically significant for changes in plasma PLP ($P = 0.0285$) but not for changes in plasma PPi ($P = 0.0715$). Results were similar between the 2 asfotase alfa dose groups (0.3 and 0.5 mg/kg/d; data not shown).

After the first 6 months, the control group (n = 6) transitioned to asfotase alfa treatment, with the last assessment before treatment initiation considered as Baseline. When analyzing the within subject data for all patients who were treated during the extension phase (n = 19), significant ($P < 0.05$) reductions from Baseline in plasma PLP and PPi
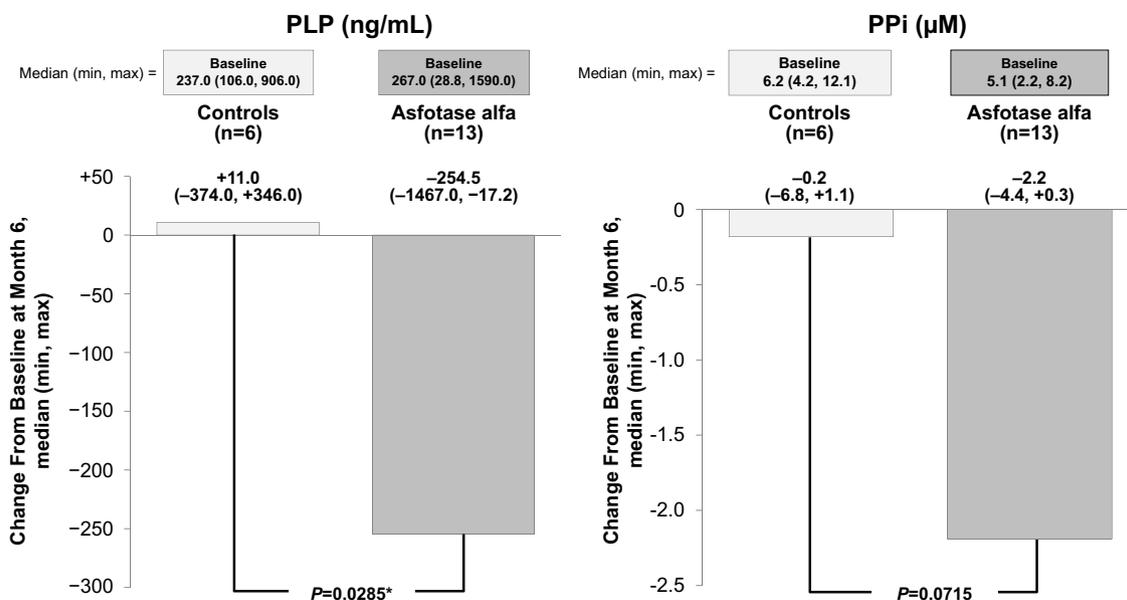
PLP (ng/mL)

Median (min, max) =

Baseline 237.0 (106.0, 906.0)	Baseline 267.0 (28.8, 1590.0)
Controls (n=6)	**Asfotase alfa (n=13)**

PPi (μM)

Median (min, max) =

Baseline 6.2 (4.2, 12.1)	Baseline 5.1 (2.2, 8.2)
Controls (n=6)	**Asfotase alfa (n=13)**

Fig. 3. Coprimary efficacy measure: median change from Baseline to Month 6 of the primary treatment period in (a) plasma PLP concentration and (b) plasma PPi concentration in patients treated with asfotase alfa versus the control group.

*P value based on exact Wilcoxon rank-sum test comparing the 2 groups.

PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

concentrations were observed at 6 months of treatment and maintained through 5 years (Fig. 4).

Planned subgroup analyses based on patient age showed that the adults ($\geq$18 years of age) had significant reductions in plasma concentrations of PLP ($P = 0.049$) and PPi ($P = 0.028$) from Baseline to Month 6 in the combined asfotase alfa group (n = 10) versus the control group (n = 3). In adolescents (13–16 years of age), reductions in plasma PLP and PPi from Baseline to Month 6 were not significant ($P > 0.05$) between the combined asfotase alfa group (n = 3) and the control group (n = 3).

3.2.2. Secondary efficacy measures
3.2.2.1. Bone mineralization: transiliac bone biopsy.
Bone histomorphometry was performed at Baseline and Year 1 in the asfotase alfa group and at Baseline and Month 6 in the control group. Our assessment (Shriners Hospital for Children, Montreal, Quebec, Canada) showed the Baseline mean (SD) osteoid volume per bone volume was 6.5% (3.9) in the combined asfotase alfa group (n = 13) and 11.6% (4.5) in the control group (n = 6). Mean osteoid volume per bone volume decreased at Year 1 in the asfotase alfa group (mean [SD] change from Baseline: -0.8% [3.4]; n = 12) and increased at Month 6 in the control group ($+0.2\%$ [4.8]; n = 6).

For comparison with the healthy population, the data were analyzed as % of healthy mean (described in Methods), with a score of 100% indicating no difference from the healthy population. Baseline mean (SD) % of healthy mean osteoid volume per bone volume was 386.1% (238.8) in the combined asfotase alfa group and 548.7% (215.3) in the control group; mean (95% CI) changes from Baseline for either of these groups (combined asfotase alfa: -103.1% [-310.0, 103.9]; control: -10.8% [-353.8, 332.2]) were not statistically significant. Baseline mean (SD) % of healthy mean osteoid thickness was 108.9% (35.4) in the combined asfotase alfa group and 178.5% (57.2) in the control group; mean (95% CI) changes from Baseline for either of these groups (combined asfotase alfa group: -0.5% [-39.3, 38.4]; control: -11.4% [-136.2, 113.4]) were not statistically significant. Baseline mean (SD) % of healthy mean mineralization lag time was 891% (623) in the combined asfotase alfa group and 530% (192) in the control group. Percent of healthy mean mineralization lag time decreased from

Baseline to Year 1 by a mean (95% CI) of -580% (-101, -452) in the asfotase alfa group, which represents a significant change ($P < 0.05$), and increased from Baseline to Month 6 by 349% (-590, 1288) in the control group, which was not a statistically significant change.

3.2.2.2. Bone mineral density: DXA.
In the 6-month primary treatment period, no statistically significant differences between treated and control patients were observed in mean (SD) changes from Baseline for the BMD Z-scores for the lumbar spine (combined asfotase alfa: $+0.2$ [0.2]; control: -0.02 [0.3]), total hip (combined asfotase alfa: $+0.1$ [0.1]; control: $+0.3$ [0.2]), or whole body (combined asfotase alfa: -0.1 [0.2]; control: $+0.2$ [0.6]). When data from the primary treatment period and the extension phase were combined, mean lumbar spine, total hip, and whole body Z-scores were within the normal range at Baseline. Significant ($P < 0.05$) changes from Baseline were observed at various time points over 5 years of treatment; however, BMD Z-scores remained within the normal range for the most part and did not change over time (Fig. 5). In patients older than 50 years of age (2 men and 8 women), mean T-scores for the lumbar spine, total hip, and whole body assessments were normal at Baseline (range: $+1.1$ to $+3.2$) and through up to 5 years of treatment (range at 5 years: -0.1 to $+1.7$).

3.2.2.3. Walking ability: 6MWT.
All randomized patients attempted the 6MWT at Baseline. In the 6-month primary treatment period, only 4 of 6 control patients had Baseline and Month 6 assessments compared with all 13 asfotase alfa–treated patients (1 control patient had no post-Baseline assessments because of cognitive impairment and limited mobility, and 1 did not have a Month 6 assessment because he was recovering from a motor vehicle accident). The median (min, max) distance walked during the 6MWT was greater by 35 (-2, 182) meters in the treated group (n = 13) and decreased by 7 (-46, 113) meters in the control group (n = 4) at Month 6. The between-group difference was not statistically significant ($P = 0.13$).

When data from the primary treatment period and the extension phase were combined, the median distance walked increased from 355 (10, 620; n = 19) meters before initiating treatment to 450 (280, 707; n = 13) meters at 5 years of treatment (Fig. 6a). The increase from

a

PLP

Plasma Concentration of PLP, median (min, max), ng/mL

Dose increases per protocol amendment

267.0 (28.8, 1590.0)

96.1 (6.3, 367.0) *

39.9 (2.5, 419.0) *

21.9 (2.7, 192.0) *

14.1 (2.5, 96.7) *

12.1 (2.5, 38.4) *

9.6 (2.5, 63.5) *

Age 5–18 y: 61.15 ng/mL — Upper normal limit
Age >18 y: 26.70 ng/mL

Age 5–18 y: 5.74 ng/mL — Lower normal limit
Age >18 y: 2.81 ng/mL

Treatment Duration[a]

	Baseline n=19	Month 6 n=18	Year 1 n=17	Year 2 n=19	Year 3 n=17	Year 4 n=15	Year 5 n=16
Mean (SD):	424.0 (423.3)	83.6 (102.8)	116.4 (113.9)	32.3 (42.5)	20.9 (21.8)	13.2 (9.4)	16.1 (15.7)
95% CI for mean:	(220.0, 628.0)	(32.4, 134.7)	(57.8, 174.9)	(11.8, 52.8)	(9.7, 32.1)	(8.0, 18.4)	(7.7, 24.5)
Change from Baseline:							
Mean		−318.4	−342.8	−391.7	−436.0	−424.2	−427.3
95% CI for mean		(−521.6, −115.2)	(−542.2, −143.4)	(−594.4, −189.0)	(−658.2, −213.8)	(−679.7, −168.8)	(−666.6, −188.0)
Median		−149.1	−210.0	−248.8	−314.7	−255.1	−284.1
(min, max)		(−1467.0, −1.1)	(−1411.0, −2.0)	(−1573.6, −24.3)	(−1576.6, −23.6)	(−1577.0, −24.9)	(−1579.6, −25.4)

b

PPi

Plasma Concentration of PPi, median (min, max), μM

Dose increases per protocol amendment

Age >18 y: 5.82 μM — Upper normal limit
Age 13–18 y: 4.78 μM

5.3 (2.2, 8.2)

3.9 (1.6, 6.8) *

3.4 (0.8, 6.0) *

2.6 (0.9, 10.9) *

2.0 (1.2, 4.6) *

2.3 (1.1, 4.5) *

2.2 (0.9, 16.0) *

Age >18 y: 1.00 μM — Lower normal limit
Age 13–18 y: 0.75 μM

Treatment Duration[a]

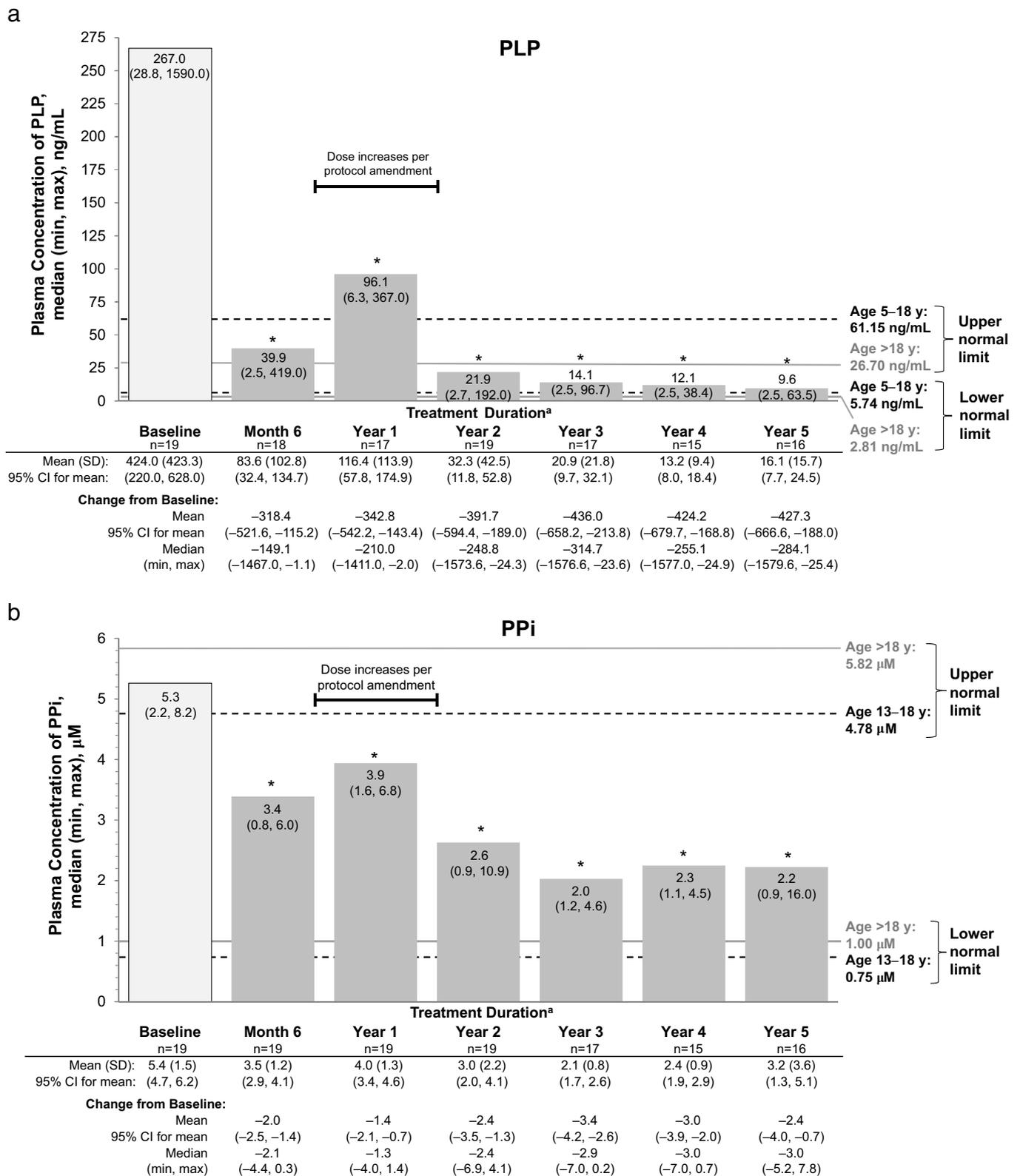|	Baseline n=19	Month 6 n=19	Year 1 n=19	Year 2 n=19	Year 3 n=17	Year 4 n=15	Year 5 n=16
Mean (SD):	5.4 (1.5)	3.5 (1.2)	4.0 (1.3)	3.0 (2.2)	2.1 (0.8)	2.4 (0.9)	3.2 (3.6)
95% CI for mean:	(4.7, 6.2)	(2.9, 4.1)	(3.4, 4.6)	(2.0, 4.1)	(1.7, 2.6)	(1.9, 2.9)	(1.3, 5.1)
Change from Baseline:							
Mean		−2.0	−1.4	−2.4	−3.4	−3.0	−2.4
95% CI for mean		(−2.5, −1.4)	(−2.1, −0.7)	(−3.5, −1.3)	(−4.2, −2.6)	(−3.9, −2.0)	(−4.0, −0.7)
Median		−2.1	−1.3	−2.4	−2.9	−3.0	−3.0
(min, max)		(−4.4, 0.3)	(−4.0, 1.4)	(−6.9, 4.1)	(−7.0, 0.2)	(−7.0, 0.7)	(−5.2, 7.8)

Fig. 4. Median (a) plasma PLP concentrations and (b) plasma PPi concentrations spanning 5 years of asfotase alfa treatment. Data from the primary treatment period and extension phase are combined. Of 18 asfotase alfa dose increases, 14 occurred approximately at or after 1 year of treatment with asfotase alfa.
[a]Time points are from the start of treatment with asfotase alfa. The control group began treatment 6 months after the treated group. Baseline for all analyses was the last assessment before the first dose of asfotase alfa.
*P < 0.05 (95% CI for mean change from Baseline did not include 0).
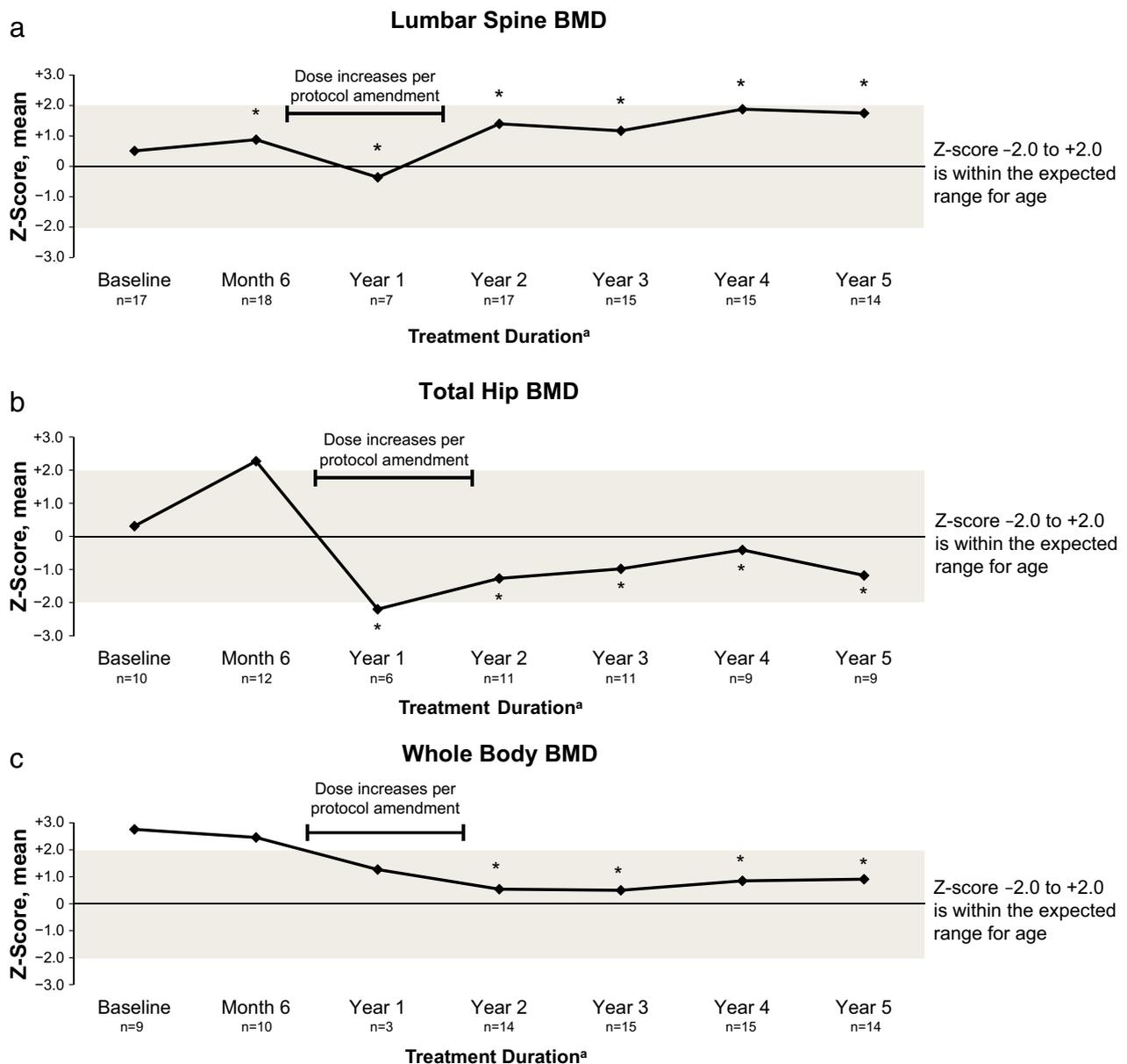PLP = pyridoxal 5′-phosphate; PPi = inorganic pyrophosphate.

Fig. 5. DXA mean (a) lumbar spine, (b) total hip, and (c) whole body Z-scores spanning 5 years of asfotase alfa treatment. Data from primary treatment period and extension phase are combined. Of 18 asfotase alfa dose increases, 14 occurred approximately at or after 1 year of treatment.

[a]Time points are from the start of treatment with asfotase alfa. The control group began treatment 6 months after the treated group. Baseline for all analyses was the last assessment before the first dose of asfotase alfa.

*$P < 0.05$ (95% CI for mean change from Baseline did not include 0).

BMD = bone mineral density.

Baseline was statistically significant at Month 6 and Years 1, 2, and 3 ($P < 0.05$; Fig. 6a). Distance walked varied widely among patients (Supplemental Fig. 1).

The median % predicted distance walked was below normal (< 84%) at Baseline (76%; n = 15), but improved to within the normal range by 6 months of treatment (85%; n = 16) and was sustained at 88% (n = 11) at 5 years of treatment in the combined asfotase alfa group (Fig. 6b). The increase from Baseline was statistically significant ($P < 0.05$) at Month 6 and Years 1, 2, 3, 4, and 5 (Fig. 6b).

Use of assistive ambulatory devices was reported in the investigator's notes for 5 of the 19 patients who attempted the 6MWT at Baseline (2 in the control group, 2 in the asfotase alfa 0.3 mg/kg/d group, and 1 in the asfotase alfa 0.5 mg/kg/d group). One patient initially in the control group was able with treatment to transition from a wheelchair to intermittent reliance on crutches by the assessments at

Year 1 and Year 1.5 but was not able to perform the assessment at Year 4 because of pain. A second patient initially in the control group used a wheeled walker through Year 1.5 and with treatment did not require its use at both Year 2 and Year 2.5, but compliance with study procedures was poor and at both visits the assessment was not completed for the full duration. Three patients maintained a reduction in reliance on assistive devices: 1 patient in the 0.3 mg group used a cane for the first 2 years, and no further use was reported through the last assessment at Year 4.5; 1 patient in the 0.3 mg group used a cane at Baseline, and no further use of a cane was reported from Month 3 through the last assessment at Year 5.5; and 1 patient in the 0.5 mg group improved from use of a wheeled walker to intermittent reliance on a cane from Year 2 through the last assessment at Year 6.
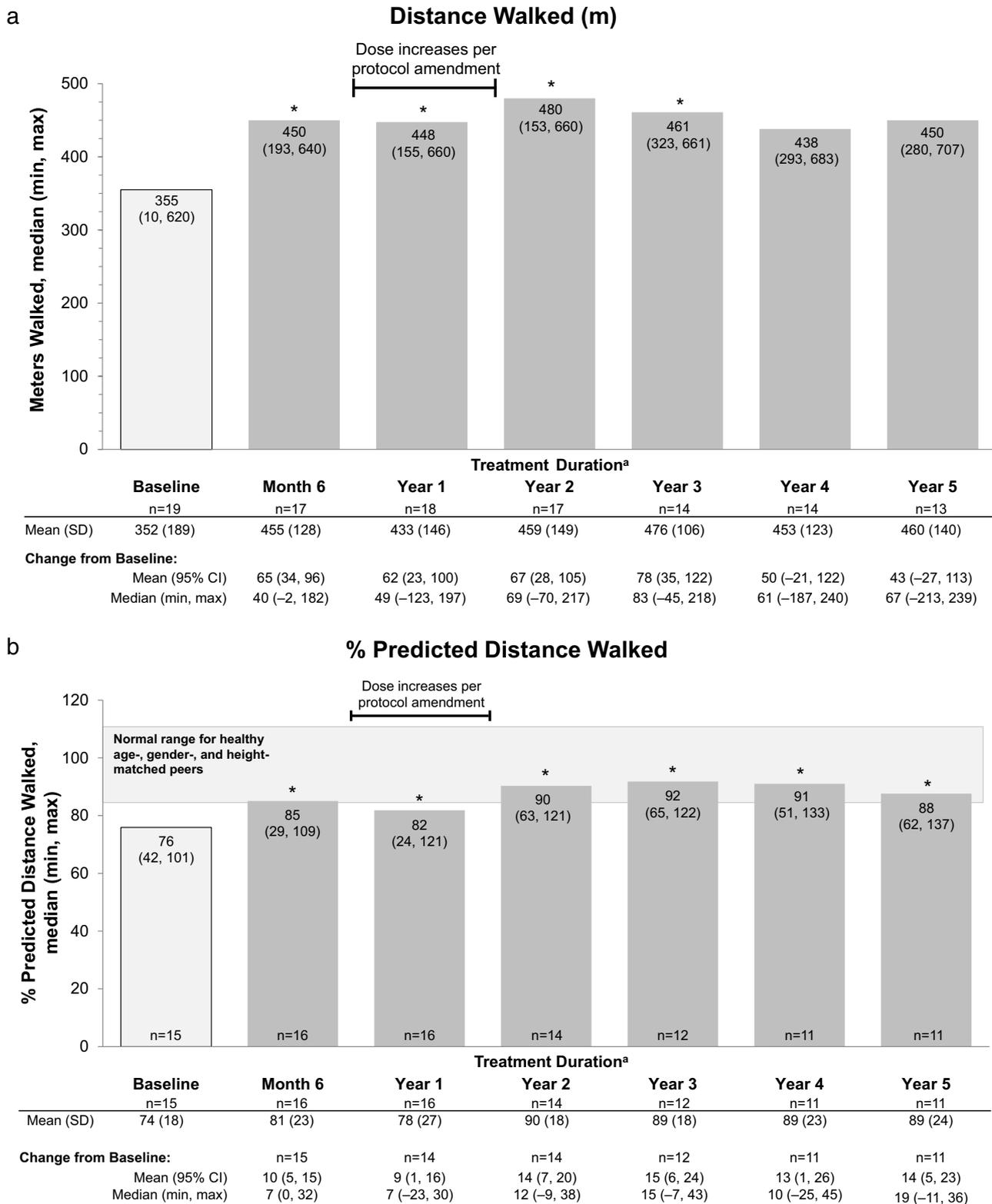
a

Distance Walked (m)

b

% Predicted Distance Walked

Fig. 6. Median (a) distance walked and (b) % predicted distance walked during the 6MWT spanning 5 years of asfotase alfa treatment. Data from primary treatment period and extension phase are combined. Of 18 asfotase alfa dose increases, 14 occurred approximately at or after 1 year of treatment. The % predicted was calculated only if the patient walked the full 6 minutes. Three patients initially assigned to the control group were not included in the % predicted analysis because they could not walk the full 6 minutes at Baseline because of physical and/or cognitive impairment; 1 additional patient was not included because she was older (66 years old) than the cutoff for calculation (65 years).

[a]Time points are from the start of treatment with asfotase alfa. The control group began treatment 6 months after the treated group. Baseline for all analyses was the last assessment before the first dose of asfotase alfa.

*P < 0.05 (95% CI for mean change from baseline did not include 0).

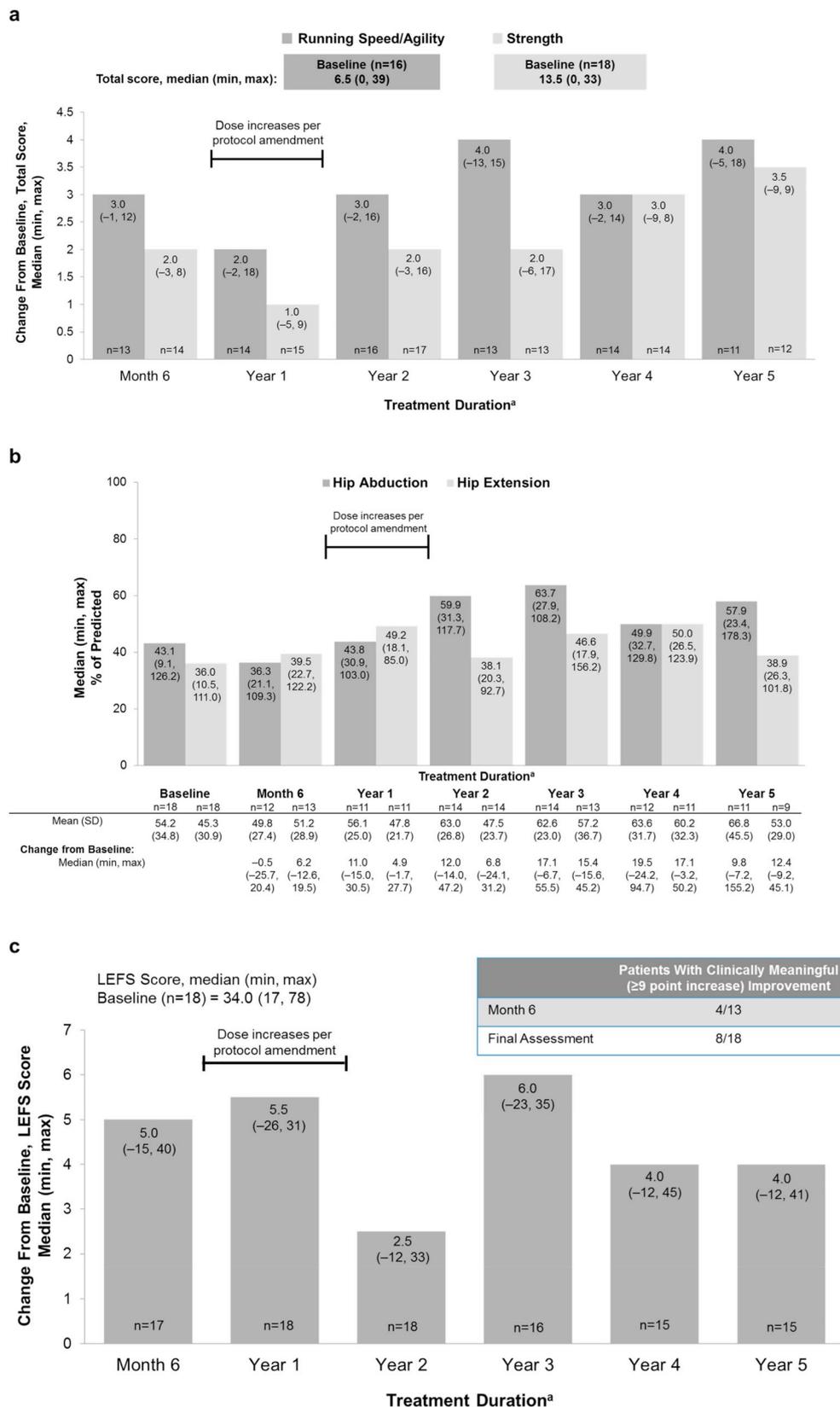6MWT = 6-Minute Walk Test.

a

b

c

Fig. 7. Median change from Baseline in BOT-2 scores for Running Speed and Agility and Strength subtests (a), median % of predicted HHD values for hip abduction and hip extension (b), and median change from Baseline in LEFS scores (c) spanning 5 years of asfotase alfa treatment. Data from primary treatment period and extension phase are combined. Changes from Baseline in the BOT-2, HHD and LEFS measures were not subject to hypothesis testing because these were exploratory efficacy measures. Of 18 asfotase alfa dose increases, 14 occurred approximately at or after 1 year of treatment.

[a]Time points are from the start of treatment with asfotase alfa. The control group began treatment 6 months after the treated group. Baseline for all analyses was the last assessment before the first dose of asfotase alfa.

BOT-2 = Bruininks-Oseretsky Test of Motor Proficiency, second edition; HHD = handheld dynamometry; LEFS = Lower Extremity Functional Scale.

3.2.3. Exploratory efficacy measures

3.2.3.1. Gross motor function: BOT-2.

From Baseline to Month 6 concluding the primary treatment period, median total (min, max) scores on the BOT-2 Running Speed and Agility subtest increased by 4 points (−1, 12) in the combined asfotase alfa group (n = 11), indicating better performance, and decreased by 0.5 points (−1, 0) in the control group (n = 2). The median total scores on the Strength subtest increased by 3 points (−2, 8) in the asfotase alfa group and by 4

points (1, 7) in the control group. After 5 years of treatment with asfotase alfa, the median changes from Baseline were 4 points in total Running Speed and Agility score (n = 11) and 3.5 points in total Strength score (n = 12), indicating improvement (Fig. 7a).

3.2.3.2. Muscle strength: HHD. Some improved strength in the proximal muscles of the hip was observed with treatment (Fig. 7b). After 5 years of treatment, median changes from Baseline in % predicted hip extension and hip abduction were 12.4 (n = 9) and 9.8 (n = 11), respectively.

3.2.3.3. Patient-reported functional disability: LEFS. In the 6-month primary treatment period, clinically meaningful improvements in LEFS scores (≥ 9-point increase) were observed for 4 of 13 (31%) patients in the asfotase alfa group and for 1 of 5 (20%) in the control group.

Over the extension phase, 14 of 18 patients (78%) with Baseline data had increases (i.e., improved) in LEFS scores at the last assessment, whereas 4 (22%) had either no change or decreased scores. For 7 of these 18 (39%) patients, the changes represented clinically meaningful improvements at the last assessment (Fig. 7c).

3.2.4. Patient-reported pain: BPI-SF

At Baseline, the median (min, max) BPI-SF total pain severity score was 15.0 (0, 30) in the combined asfotase alfa group (n = 13) and 12.0 (5, 25) in the control group (n = 6). In the primary treatment period, changes from Baseline to Month 6 were similar between the treated (−2.0 [−17, 4]; n = 13) and control groups (−3.0 [−11, 1]; n = 4). BPI-SF scores improved over the extension period, with a median (min, max) decline from Baseline of −1.0 (−21, 8; n = 19) at Year 1 and −3.5 (−20, 5; n = 16) after up to 5 years of treatment.

3.3. Safety

No deaths occurred during the study. All patients experienced ≥ 1 treatment-emergent AE (TEAE); most TEAEs were mild (864/1145 [75%]) or moderate (229/1145 [20%]) in intensity. Table 2 summarizes the TEAEs reported in > 3 patients. The most common TEAEs were ISRs (385/1145 [34%]), which occurred in all patients. The most common ISRs (≥ 5 patients) were erythema (13/19 [68%]), hematoma (10/19 [53%]), skin discoloration (9/19 [47%]), ISR not otherwise specified (7/19 [37%]), pain (6/19 [32%]), atrophy (5/19 [26%]), and pruritus (5/19 [26%]). Two patients experienced TEAEs categorized as hypersensitivity IARs, oral hypoesthesia and chills in 1 patient and anaphylactoid reaction in 1 patient (see Supplemental materials); each was considered moderate in intensity. The patient who had the anaphylactoid reaction withdrew from the study. In 1 patient, the dose of asfotase alfa was reduced (from 0.5 mg/kg/d 7 times per week to 0.5 mg/kg/d 3 times per week) because of ISRs (discoloration, atrophy, and pruritus).

A total of 29 treatment-emergent SAEs were reported for 9 patients. Eight events in 2 patients were assessed by the investigator as related to study drug (oral hypoesthesia, chills, pain in extremity, and headache in 1 patient and hypersensitivity reaction and anaphylactoid reaction in 1 patient).

Eleven patients had 22 events of injection site lipodystrophy, none of which were SAEs. All of these events were ongoing at the end of the study except for 3 events in 2 patients (2 events of hypertrophy in both arms of 1 patient resolved after ~17 months and 1 event of stretched skin at the umbilicus resolved after ~16 months).

Two patients had ocular calcifications noted on ophthalmologic/funduscopic exam at Baseline, which is consistent with the natural history of HPP. Nine patients (13–66 years of age) had treatment-emergent ocular calcifications located either at the conjunctiva or corneal limbus, none of which were considered SAEs. These calcifications were first observed after ≥ 72 weeks of treatment in all but 2

patients. None of these 9 patients had ocular calcifications noted at Baseline. All ocular calcifications were considered mild in severity, did not worsen during the study, and did not affect vision or ocular structure. Of the 9 patients, 2 underwent conjunctival biopsy that showed epithelial-subepithelial junction calcification and actinic elastosis (Supplemental Fig. 2). All these events were considered by the investigator to be possibly (9 events) or probably (2 events) related to study drug.

One patient had nephrocalcinosis noted at Baseline, which is consistent with early-onset, severe hypophosphatasia. Two patients (1 adult and 1 adolescent) had 3 TEAEs of nephrocalcinosis identified by renal ultrasound, as assessed routinely by the study site radiologist(s), after 22 weeks of treatment or longer. Neither of these patients had renal calcification on the Baseline ultrasound. The investigators considered all events to be mild in severity; 1 event was considered possibly related to study drug, and 2 were considered unlikely related or unrelated to study drug.

Eleven patients had a total of 25 fractures reported as TEAEs; none were considered related to treatment.

Laboratory assessments of serum ALP activity for the entire treated cohort showed that, as expected, ALP activity was markedly increased with asfotase alfa treatment (Supplemental Fig. 3). Median ALP activity peaked at 6819 U/L (range: 3047–12,630; n = 13) at 4 years after the dose was increased to 6 mg/kg/wk. At final assessment (Year 5), median ALP activity was 3154 U/L (range: 803–9917; n = 19).

Seventeen of 19 (89.5%) patients tested positive for anti–asfotase alfa antibodies. Three patients had peak titers during treatment that were considered high (> 128), with a maximum titer of 512. No patient had a high titer at the last assessment. Four of the 17 (23.5%) patients with positive anti–asfotase alfa antibody results tested positive for neutralizing antibodies (in vitro assay % inhibition: > 4.5%) during treatment (range: 4.6% to 5.9%), but none tested positive for neutralizing antibodies at the last assessment.

4. Discussion

This Phase 2, dose-ranging study is the first to assess the efficacy and safety of asfotase alfa initiated in adults and adolescents with HPP, nearly all of whom had the infantile or childhood forms of HPP. The coprimary efficacy measure of change in plasma PLP concentration was met, with significant decreases in plasma PLP observed in treated versus control patients at Month 6. However, statistical significance of the coprimary efficacy measure of change in PPi concentration during the 6-month primary treatment period was not met. Notably, the dose of asfotase alfa (2.1–3.5 mg/kg/wk) administered during the 6-month primary treatment period, and initially in the open-label extension phase, was lower than the dose administered in later investigations of asfotase alfa in perinatal, infantile, and childhood forms of HPP (6 mg/kg/wk) [41,42]. For most patients in the current study, asfotase alfa reduced plasma concentrations of PLP and PPi to within the normal range, maintaining normal substrate concentrations through study end, consistent with its expected biologic action [24] and previous studies in infants and children with HPP [25,27]. Circulating levels of these substrates typically remained within the normal range despite high ALP activity when patients were receiving asfotase alfa treatment (Supplemental Fig. 3). Pharmacodynamic results in the primary treatment period were similar in the 2 asfotase alfa dose groups (0.3 and 0.5 mg/kg/d).

Importantly, the decreases in circulating TNSALP substrates were associated with improved functional measures in treated patients at the end of the 6-month primary treatment period. In the extension phase, a protocol amendment allowed the dose to be increased to 6 mg/kg/wk, comparable to the dose showing effectiveness in children [27]. Increasing the dose of asfotase alfa was associated with further reductions in TNSALP substrates and seemed to be associated with additional improvements in functional measures. TNSALP substrates generally stayed within the normal range during treatment with the higher dose,

Table 2
TEAEs occurring in > 3 patients during the study.

TEAE (preferred term)	Patients, n (%) N = 19
Arthralgia	13 (68)
Injection site erythema	13 (68)
Pain in extremity	12 (63)
Back pain	10 (53)
Injection site hematoma	10 (53)
Bone pain	9 (47)
Injection site discoloration	9 (47)
Musculoskeletal pain	8 (42)
Peripheral edema	8 (42)
Dizziness	7 (37)
Eye deposit	7 (37)
Foot fracture	7 (37)
Injection site reaction not otherwise specified	7 (37)
Joint swelling	7 (37)
Upper respiratory tract infection	7 (37)
Headache	6 (32)
Injection site pain	6 (32)
Nasopharyngitis	6 (32)
Cough	5 (26)
Fall	5 (26)
Injection site atrophy	5 (26)
Injection site pruritus	5 (26)
Procedural pain	5 (26)
Sinusitis	5 (26)
Fatigue	4 (21)
Injection site hypertrophy	4 (21)
Injection site swelling	4 (21)
Nausea	4 (21)
Oropharyngeal pain	4 (21)
Osteoarthritis	4 (21)
Paraesthesia	4 (21)
Post-traumatic pain	4 (21)

TEAE = treatment-emergent adverse event.

suggesting that it was not excessive.

At Baseline, the adults and adolescents with HPP enrolled in this study had elevations in osteoid volume per bone volume and mineralization lag time, consistent with the impaired skeletal mineralization of this disorder [21]. Baseline values of osteoid thickness were more variable, with higher values in the control group than the combined asfotase alfa group. The only significant change from Baseline was observed for mineralization lag time in the treated group at Year 1. Interpretation of these biopsy data was, however, perhaps limited by the study design as biopsy results from the previous Phase 1 investigation were used as Baseline values for some patients, resulting in variability in the time between biopsies. Furthermore, biopsies were conducted after treatment with low-dose asfotase alfa (2.1–3.5 mg/kg/wk), not after the dose was increased to 6 mg/kg/wk.

There were no clear differences between treated and control patients in DXA results at the end of the 6-month primary treatment period. When data from the primary treatment period and extension phase were combined, mean lumbar spine and total hip BMD Z-scores were within normal range at Baseline; the mean values for whole body BMD were somewhat elevated early on and then fell into the normal range [31]. Although treated patients showed changes from Baseline in lumbar spine, total hip, and whole body BMD Z-scores that persisted through the extension phase, mean scores remained mostly within the normal range, with no suggestion of excessive mineralization with treatment. In clinical practice, Z-scores are generally recommended for women prior to menopause and in men younger than 50 years of age [31]. In our patients older than 50 years of age, mean T-scores were normal at Baseline and through up to 5 years of treatment.

Normal or above normal DXA BMD measurements in HPP patients have been noted by other investigators as well [43,44]. The explanation is unclear, but it has been postulated that increased bone tissue from osteomalacia or distorted bone trabeculation with areas of decreased

mineralization and areas of local hypermineralization (sclerosis) may impact DXA results in HPP patients [45]. As such, DXA BMD measurements may be confusing for the assessment of disease severity and treatment response in patients with HPP.

Patients with HPP often have poor strength and compromised physical function that can manifest as decreased ambulatory ability among other functional problems [14,16,17]. In the web-based Hypophosphatasia Impact Patient Survey (HIPS) and the Hypophosphatasia Outcomes Study Telephone interview (HOST) of 125 adults with HPP, 60% reported use of an ambulatory assistive device at some time (e.g., wheelchairs, walkers, canes, orthotics) [14]. In the current study, patients had a wide spectrum of functional capabilities at Baseline, ranging from normal ambulation to nonambulatory. Although considerable variability of response concerning functional measures was demonstrated among the treated patients, several of them experienced clinically meaningful improvements in mobility. As detailed in Supplemental Fig. 1, of the 18 patients with a Baseline and post-Baseline assessment, 14 (78%) improved in distance walked. The % predicted distance walked on the 6MWT for the entire treatment group improved from below normal (< 84%) to within the normal range for healthy peers at Month 6, and was sustained through Year 5. Median changes in distance walked at Years 1, 2, 3, 4, and 5 (range: 49–83 m) exceeded the MCID for adults and adolescents with pediatric-onset HPP of 31 m and 43 m, respectively [35]. Further, although information concerning use of ambulatory devices was not formally collected in the study, investigator notes indicated that 5 patients used assistive devices (wheelchair, walker, crutches, or cane) while attempting the 6MWT at Baseline. At study end, 2 patients no longer relied on assistive devices and 1 patient improved from reliance on a walker to use of a cane. Improvements were also recorded for some patients in gross motor function (BOT-2 scores), proximal muscle strength (HHD assessments), and patient-reported functional disability (LEFS scores). Improved mobility might enable some patients to engage in new activities, leading to accidents after prolonged periods of immobilization, and confounding assessments of functional improvements.

This study has several limitations. The coprimary efficacy measure (change from Baseline in PLP and PPi at Month 6) was not met, possibly because of small sample sizes. Furthermore, study patients received lower doses of asfotase alfa (2.1–3.5 mg/kg/wk) during the primary treatment period and in the beginning of the extension phase than later in the extension phase. Although no statistical analyses were performed during the extension phase to assess the effect of increasing the dose to the currently indicated 6 mg/kg/wk, the doses in the primary treatment period were possibly suboptimal for decreasing the TNSALP substrate concentrations, shown by the biochemical data (PLP, PPi). A later study assessing the pharmacodynamics and pharmacokinetics of asfotase alfa in adults with pediatric-onset HPP demonstrated that the doses of 6 and 9 mg/kg/wk provide significantly greater reductions in PPi than 1.5 mg/kg/wk [46], confirming the efficacy of doses higher than those initially evaluated in the current study. It should also be noted that, at this time, assays for PPi are not commercially available. In addition, the study population with this rare disorder was heterogeneous with interindividual differences that may have influenced the results, including age at Baseline, age at onset of signs or symptoms of HPP, and genetic background. There were imbalances between the control and treatment groups with regard to some Baseline characteristics such that the control group may have had more substantial disease burden. All patients in the control group had a medical history of fracture at Baseline, despite their younger age (median: 21 vs. 55 years in the combined asfotase alfa group); 3/6 were unable to perform the 6MWT at Baseline because of physical and/or cognitive impairment, and 5/6 had compound heterozygous *ALPL* mutations (compared with 5/13 in the combined asfotase alfa group with a single allele mutation). Lastly, given the small sample size, results of prespecified subgroup analyses comparing adults with adolescents were inconclusive, and the low number of adolescents enrolled in the study does not allow for generalizability of the data to this age group.

As mentioned, although standardized protocols were provided and surgeons performing bone biopsies had such experience, interpretation of the bone biopsy and perhaps DXA results (both secondary efficacy measures) was limited by varying techniques across sites. The study was also not powered to show differences in secondary or exploratory measures. Some of the functional outcome scales used may not have been appropriate for the study population. For example, the BOT-2 is validated only for ages 4–21 years [36]. Because the median age of patients with HPP in this study at enrollment was 53 years, only total scores were used rather than age-standardized scores.

Asfotase alfa was generally well tolerated over 5 years of treatment. No patient deaths occurred during the study. Two patients had hypersensitivity reactions; the patient who experienced oral hypoesthesia and chill resumed asfotase alfa therapy without further reactions, and the patient who had an anaphylactoid reaction withdrew from the study. TEAEs were generally mild or moderate in intensity and were most commonly ISRs. The risk of ISRs such as lipohypertrophy and injection site atrophy can be reduced by rotating injection sites between the abdominal, deltoid, and thigh areas [47]. Asymptomatic conjunctival calcifications occurred after initiating treatment in 9 patients, with no effect on vision or the anatomic integrity of the eye. Ophthalmologic examinations were not standardized in this study and some of the exams conducted post-treatment were more detailed than those conducted at Baseline. Hence, some calcifications may have been missed at Baseline. It is unclear whether these ectopic calcifications observed in patients with HPP treated with asfotase alfa are a consequence of the disease or related to treatment. Anti–asfotase antibodies were detected in 17 patients, 4 of whom transiently tested positive for neutralizing antibodies during treatment. There was no certain evidence of a clinically relevant effect on efficacy or safety, such as loss of efficacy, hypersensitivity, or need for dose change, in these 4 patients.

4.1. Conclusions

Consistent with its intended biologic action, asfotase alfa treatment in adults and adolescents with HPP decreased plasma concentrations of PLP and PPi to levels within normal reference ranges by Month 6. When comparing the treatment group and the control group at Month 6, reduction in PLP reached statistical significance while the numerical decreases in PPi did not. Levels of these ALP substrates were maintained within the normal range through 5 years of therapy. Treatment with asfotase alfa was generally well tolerated, with ISRs being the most common TEAE. Although statistical significance on the prespecified coprimary efficacy measure (reductions in PLP and PPi at Month 6) was not met, the totality of the data indicates efficacy of asfotase alfa in the treatment of this older population of patients with HPP.

Author roles

Study design: Michael P. Whyte; Priya S. Kishnani; Cheryl Rockman-Greenberg; Scott Moseley; Andrew Denker; Eric Watsky

Study investigator: Priya S. Kishnani; Cheryl Rockman-Greenberg;

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Enrolled patients: Priya S. Kishnani; Cheryl Rockman-Greenberg; Michael P. Whyte

Collection and assembly of data: Priya S. Kishnani; Cheryl Rockman-Greenberg; Michael P. Whyte; M. Tariq Bhatti

Data analysis: Scott Moseley; Andrew Denker; Eric Watsky

Data interpretation: All authors

Manuscript review and revisions: All authors

Final approval of manuscript: All authors

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.12.011.

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