Online-Only Supplement

eMethods

Study Oversight

This was a Phase 2, randomized, open-label, dose-ranging, historical control study sponsored by Alexion Pharmaceuticals. The study design was developed in collaboration between the sponsor and the principal investigators. The institutional review board (IRB) or independent ethics committee (IEC) at the two investigational sites reviewed and approved the protocol and informed consent form for the study prior to study initiation. Investigators were responsible for assuring IRB/IEC compliance with applicable regulations throughout the duration of the study. The investigation was conducted in accordance with the provisions of the Declaration of Helsinki and Good Clinical Practice guidelines as defined by the International Conference on Harmonisation. The statistical analyses for the study were performed by Alexion Pharmaceuticals. Other organizations involved in the management and analysis of study data included: Document Solutions Group, which was responsible for data management and development of the primary electronic data capture system; Biomedical Systems, which managed radiographs for radiographic evaluations; Midwest BioResearch, which performed analysis of pharmacokinetic samples; Covance, which performed blood analyses and urinalyses; Connective Tissue Gene Tests, which performed gene mutation analyses; and Pharmaceutical Product Development, LLC, which tested for anti-asfotase alfa antibodies. A Data Safety Monitoring Board, an independent panel of 3 physicians, convened on a periodic and ad hoc basis, when necessary, to perform independent review of clinical data for the study. A panel of 3 independent radiologists, experts in skeletal dysplasias, evaluated patient radiographs.

Michael P. Whyte, MD served as the primary author of the manuscript in collaboration with Cheryl Rockman-Greenberg, MD, CM and Katherine L. Madson, MD, PhD. The final draft was reviewed by all co-authors, who vouch for its accuracy.

Selection of Study Population

Inclusion criteria:

1. Patient, parent, or legally authorized guardian(s) provided informed assent/consent prior to study
2. The patient's parent or other legal guardian was willing to comply with study requirements
3. Patients ≥5 and ≤12 years of age with open growth plates at time of enrollment
4. Tanner stage of 2 or less, indicating prepubescence
5. Documented history of hypophosphatasia (HPP) as evidenced by:
    a. Presence of HPP-related rickets on skeletal radiographs
    b. Serum alkaline phosphatase (ALP) activity below the age-adjusted normal range
    c. Plasma pyridoxal 5′-phosphate (PLP) level at least twice the upper limit of normal
6. Serum 25-hydroxy [25(OH)] vitamin D level ≥20 ng/mL
7. Ability of patient and patient's parent(s) or legal guardian(s) to comply with the study protocol

Exclusion criteria:

1. History of sensitivity to any of the constituents of asfotase alfa
2. A medical condition, serious intercurrent illness, or other extenuating circumstance that, in the opinion of the investigator, may have significantly interfered with study compliance, including all prescribed evaluations and follow-up activities
3. Treatment with an investigational drug within 1 month prior to study
4. Current enrollment in any other study for HPP
5. Serum calcium (Ca^{2+}) or phosphorus level below the age-adjusted normal range
6. Current evidence of a treatable form of rickets
7. Prior treatment with bisphosphonates
8. Bone fracture or orthopedic surgery within the past 12 months that, in the opinion of the investigator, would interfere with the ability of patients to comply with protocol
9. A major congenital abnormality other than those associated with HPP

Historical Control Criteria:

1. Patients with infantile or childhood HPP who met inclusion criteria but did not qualify for treatment based on age at study initiation or otherwise declined participation.
2. Available historical radiographs from 5 to 12 years of age (±11 months) with evidence of open growth plates
3. Minimum of 2 radiographs sets (bilateral PA views of the hands/wrists and bilateral AP views of the knees) taken at least 6 months, but no more than 2 years, apart
4. Serum ALP below the age-adjusted normal range, plasma PLP at least twice the upper limit of normal, serum Ca^{2+} and phosphorus levels within age-adjusted normal range
5. No previous treatment with bisphosphonates

Dosing

Asfotase alfa was administered at the investigational site, thereafter by the parent or legal guardian (with medical supervision during the first month). Doses (concentration 40 or 100 mg in 1 mL) could be adjusted by the investigator together with the sponsor for safety concerns, tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP) substrate levels, or lack of efficacy. Following the 6-month initial phase, the extension phase was designed with a maintenance dose of 3 mg/kg/week based on calculations from animal studies and an initial Phase 1 study in adults. All children started the extension phase at this dose. Then, as early as 1.5 months afterwards, the investigators described that clinical outcomes worsened. Therefore, based on these reports and an interim analysis of other studies (1), the protocol was changed and the dose increased to 6 mg/kg/week for the ongoing extension phase of the trial.

Given that no statistical significant difference was noted between the 2 dose groups, and groups received the same dose after the initial 6 months, these and subsequent data at individual timepoints were pooled and reported as a single treatment group.

Outcome Assessments

The patients for treatment were randomized and stratified by site, with a block size of 4 with blinded allocation administered through the electronic data capture system. For biochemical testing, blood collected preprandially, if feasible, was assayed for serum ALP activity by WIL Research (Skokie, IL); plasma inorganic pyrophosphate (PPi) first by the sponsor and then by Charles River Laboratories - Preclinical Services Montreal (Senneville, Quebec, Canada); and plasma PLP first by Associated Regional and University Pathologists Laboratories (Salt Lake City, UT), then by Covance (Princeton, NJ), and subsequently by Biotrial Bioanalytical Services, Inc. (Laval, Quebec, Canada), respectively. In the extension phase, plasma PPi and PLP levels were measured before asfotase alfa injection and after a 4-hour fast, if feasible.

Table 1. **Serum Concentration Normal Reference Ranges for Metabolic Variables in This Study**

Parameter	Reference Range
ALP	See individual patient narratives for sex and age adjusted lower limits.
PPi	<0.75 – 5.71 µM
PLP	5.74 – 61.15 ng/mL
25-hydroxyvitamin D	62 – 200 nmol/L
PTH	1.05 – 6.83 pmol/L
Ca^{2+}	2.12 – 2.57 mmol/L

Growth was evaluated using stadiometer-measured height, weight, and body mass index (BMI) Z-scores, ie, standard deviations from age- and sex-matched means calculated using the Centers for Disease Control 2000 growth charts and data tables (http://www.cdc.gov/growthcharts/data_tables.htm).

Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is a norm-referenced instrument used to assess developmental functioning of children from 4 to 21 years of age, and to identify children with developmental delay (2). Raw scores of successfully completed items are converted to scaled and composite scores allowing comparison with children in an age- and sex-matched standardized sample. The distances for the 6-minute walk test (6MWT) were normalized to percent predicted for age, height, and sex using the formula from Geiger (2007) (3). The Child Health Assessment Questionnaire (CHAQ) is a parent-reported questionnaire with 8 subscales to assess function and level of disability during performance of daily activities. The Pediatric Outcomes Data

Collection Instrument (PODCI) evaluates parent-reported mobility for daily life and overall health, pain, and ability to participate in normal and vigorous daily activities typically associated with young people. PODCI scores are contrastable to the US healthy population.

Iliac crest histomorphometry and dual energy x-ray absorptiometry (DXA) evaluations are in preparation for separate detailed publications.

Individual Patient Profiles

PATIENT 1

At study entry, Patient #1 was an 8 year and 8 month-old girl. She had presented at age 1 with failure to thrive requiring gastrostomy tube placement at 3 years of age, which was later removed at age 5 years (in April 2006). She had premature deciduous tooth loss at age 15 months, rachitic rosary, knock knees, delayed onset of walking at 26 months, and a waddling gait. Sagittal suture craniosynostosis developed at 4 years of age, and a Chiari I malformation with thoracic syringomyelia, assessed not to need surgery, was noted prior to study entry. At study baseline, she could only walk 2 blocks twirling a baton before fatiguing. She had below average height (Z-score=-1.5) and weight (Z-score=-1.8). Baseline serum levels were ALP (50 U/L), PPi (4.8 μM), PLP (527 ng/mL), 25-hydroxyvitamin D (79 pmol/mL), parathyroid hormone (PTH; 1.3 pmol/L), and total Ca^{2+} (2.5 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 69 U/L. Normal reference ranges for other parameters are also presented in Table 1.

She began asfotase alfa therapy at 6 mg/kg/week given as 3 injections. Figure 1 shows the radiographic improvement of the left wrist and left knee during treatment with asfotase alfa. After 1.5 months of treatment, she had a better appetite, and her teeth were erupting.

Patient 1 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	5:1-5:1	9	4:6-4:7	4
6 months	10:0-10:2	17	9.0-9.2	15
1 year	9:6-9:8	15	8:3-8:5	13
2 years	≥19	21	NA	NA
3 years	≥19	19	≥19	24
4 years	≥19	19	≥19	21
5 years	≥19	21	≥19	22

BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scale score for healthy age- and sex-matched peers is 15 (5).

Her BOT-2 scaled scores at baseline for the Running Speed and Agility subset and the Strength subset were both below normal. During treatment, her performance scores increased for running and strength. She also demonstrated an improved ability to run and pick up an object as well as to control acceleration and deceleration during ambulation. She also showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors, extensors, hip abductors, and flexors, as measured by handheld dynamometry (HHD) (>100% predicted for normal peers). This was also demonstrated by her ability to walk longer distances (measured by the 6MWT predicted for sex-, age-, and height-matched peers), increasing from 491 meters (82% of predicted) at baseline to 603 meters (89% of predicted) at last assessment.

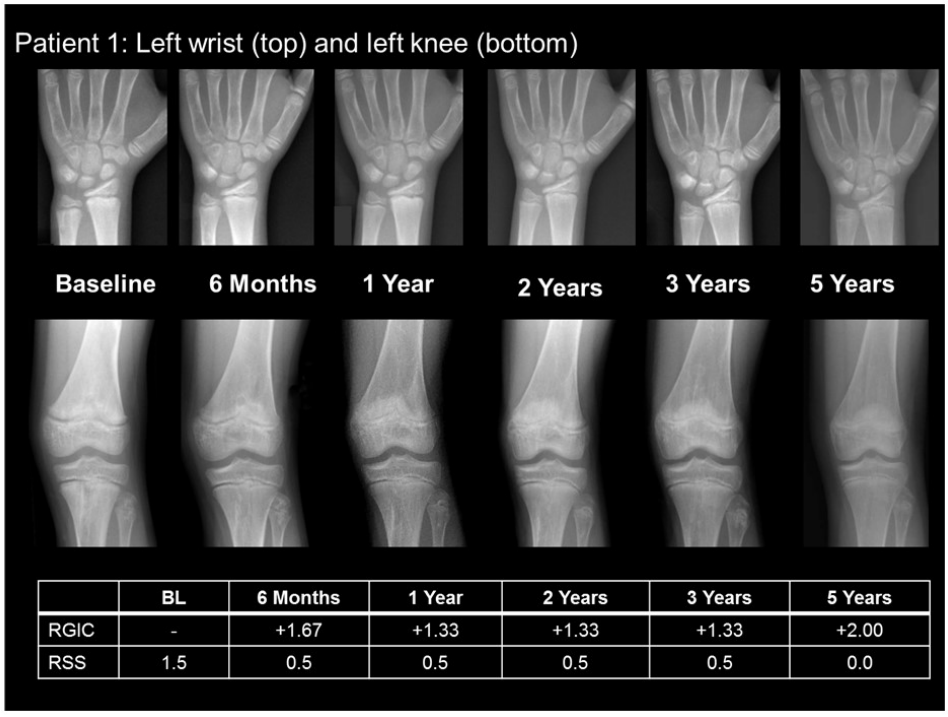
She did not experience any treatment-emergent adverse events (TEAEs) that resulted in a change in dosing regimen. She had recurrent injection site reactions (ISRs), including erythema, discoloration, macules with erythema, purplish discoloration or atrophy, beginning Day 18 up to the last visit; all were assessed as mild in severity and definitely related to study drug.

She also showed signs of subclinical calcification in her eyes as evidenced by conjunctival deposits found during each ophthalmologic examination after 2.5 years of treatment; this was assessed by the investigator as mild

and probably related to the study drug. During the 2.5-year examination, a "focal echogenic lesion in the midpole of one kidney, which may represent a tiny renal stone" was noted and persisted unchanged through Year 3 but not thereafter; it did not recur. This event was not considered clinically significant by the investigator. At almost Year 1, she fractured the right distal radius, which was considered unrelated to study drug by the investigator; the fracture healed in 2.5 months. She had no anti-asfotase alfa antibodies at baseline, but developed antibodies after 1 year of treatment, which persisted. She has not demonstrated neutralizing antibody activity *in vitro*.

Presently, at age 13 years following 5 years of asfotase alfa treatment, she participates in physical education classes without restrictions, performs gymnastics, takes dance lessons, and made the track team.

Patient 1 Radiographic Improvement in the Left Wrist and Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+1.67	+1.33	+1.33	+1.33	+2.00
RSS	1.5	0.5	0.5	0.5	0.5	0.0

PATIENT 2

Patient #2 was an 8 year and 1 month-old boy at baseline, who manifested prenatal bowing and Bowdler spurs detected by ultrasound (4) but postnatally he followed a course in keeping with childhood HPP. He left the hospital on his 2nd day of life and otherwise had a normal infancy. Premature deciduous tooth loss began at 15 months. He walked at 19 months, and at 26 months was unable to run or jump. Prior to enrolling in the study at the age of 8 years, his gait was considerably abnormal as he forcibly used his upper extremities to help propel himself. He complained of "tired muscles" and was unable to keep up with his classmates, had difficulty ascending and descending stairs, and was unable to hop or jump. He had flaring of the lower costal margins, dimples in the forearms and thighs (from the spurs), bowing of arms with loss of motion in the forearms, knocked knees accompanying his unusual gait, and muscle weakness. Epiphysiodeses had been performed at age 6 years on his lateral tibial growth plates, and were removed at age 9 years. He was short (Z-score=-3.8) but had appropriate weight for height (Z-score=-3.5). Baseline serum levels were ALP (49 U/L), PPi (6.96 µM), PLP (333 ng/mL), 25-hydroxyvitamin D (94 pmol/mL), PTH (1.5 pmol/L), and Ca^{2+} (2.6 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 86 U/L. The reference ranges of the other parameters are also presented in Table 1.

He initiated asfotase alfa treatment at 9 mg/kg/week given as 3 SC injections per week.

At 1.5 months of treatment, he played with his brother and no longer used his arms to help himself walk. His physical education teacher noticed improved running and ability to jump after 3 months of therapy.

Patient 2 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	3	4:4-4:5	6
6 months	4:1-4:1	6	6:6-6:8	10
1 year	4:1-4:1	6	6:0-6:2	8
2 years	7:3-7:5	9	8:0-8:2	11
3 years	NA	NA	NA	NA
4 years	7:3-7:5	9	9:3-9:5	11
5 years	8:0-8:2	10	12:0-12:5	15

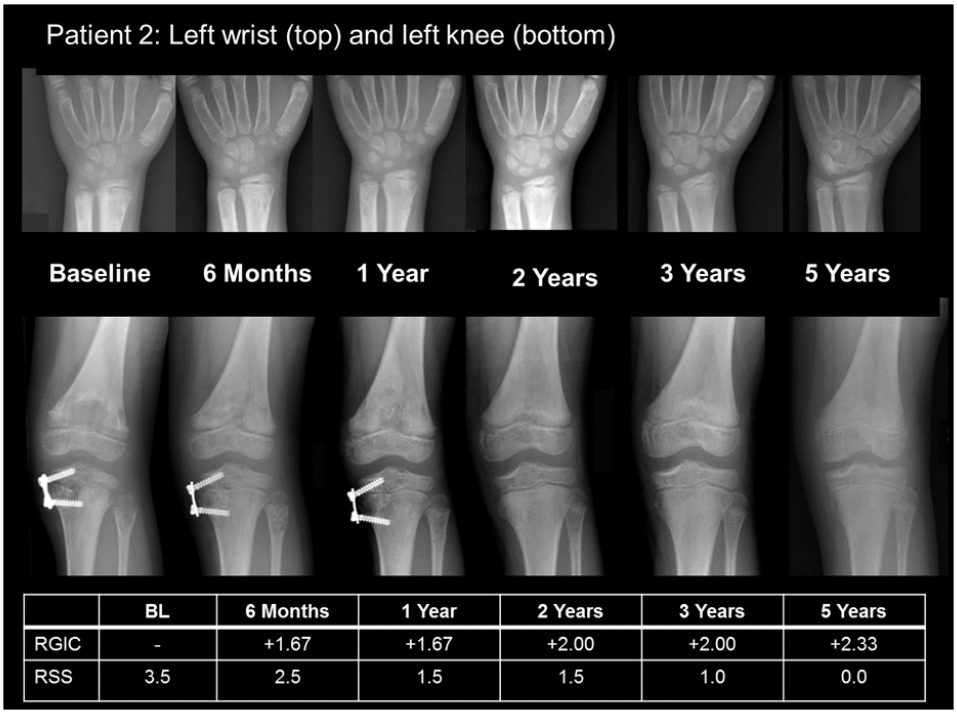
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

His BOT-2 baseline scaled scores were well below normal for the Running Speed and Agility subset and the Strength subset. During treatment, his performance scores increased significantly for running and strength, indicating that improved muscle strength facilitated skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. Also, he showed significant increases over 5 years of treatment in torque and median force for individual muscle groups including knee flexors (74%, 59% predicted [right, left]) and extensors (67%, 81%) and hip abductors (57%, 59%) and flexors (43%, 40%), as measured by HHD. This was also demonstrated by his ability to walk longer distances (6MWT); increasing from 350 meters (61% predicted) at baseline to 467 meters (70% predicted) at last assessment. His height increased from Z=-3.8 to -2.0, and weight from Z=-3.5 to -1.3, at last assessment.

Ectopic calcification, evidenced by conjunctival deposits, was found during each ophthalmologic examination after 2.5 years of treatment. He did not experience any TEAEs that resulted in dosing regimen change. Beginning on day 5 to last assessment he had recurrent ISRs, including erythema, pain, swelling, discoloration, and lipohypertrophy. All were assessed by the investigator as mild or moderate and definitely related to study drug. Asfotase alfa antibodies were detected from 1.5 months of treatment through last assessment. He was positive for neutralizing antibody activity only at 4.5 years of therapy.

Now 13 years old after 5 years of therapy, his overall health is good. He participates in physical education without restrictions and is able to keep up with his peers, shoot baskets, ride his bicycle, and jump on the trampoline.

Patient 2 Radiographic Improvement in the Left Wrist and Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+1.67	+1.67	+2.00	+2.00	+2.33
RSS	3.5	2.5	1.5	1.5	1.0	0.0

PATIENT 3

Patient #3 was a 5 year and 11 month-old boy at study entry. He was a fraternal twin who fell off the growth curve and manifested hypotonia at 5 months of age, requiring occupational and physical therapy. He did well with pureed foods, but poor muscle strength caused trouble chewing solids necessitating help from a therapist until age 3 years. Deciduous tooth loss began at 18 months. Independent walking was delayed, beginning at age 3, with only a few independent steps. He used a walker to ambulate from 2.5 to 4 years of age. An oral pathologist diagnosed infantile HPP when he was age 4.5 years. Prior to enrollment at 6 years of age, he had frequent shin and ankle pain requiring rest for relief. He could not jump, hop, or run. He was slower, weaker, and had less endurance than his twin brother. His height was in the normal range at 25% (Z-score=-0.7) and his weight at 11% (Z-score=-1.2). Baseline serum levels were ALP (68 U/L), PPi (5.5 µM), PLP (76 ng/mL), 25-hydroxyvitamin D (111 pmol/mL), PTH (1.4 pmol/L), and Ca^{2+} (2.6 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 93 U/L. Normal reference ranges of the other parameters are also presented in Table 1.

He initiated asfotase alfa therapy at 6 mg/kg/week given as 3 injections.

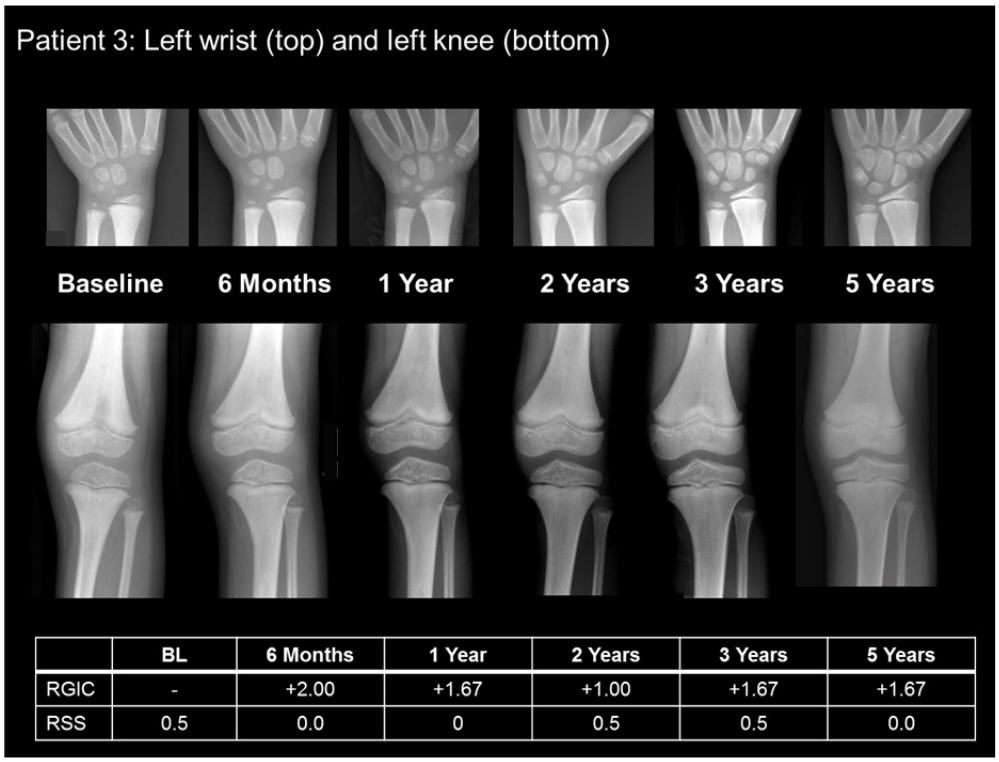
Patient 3 Functional Outcome Measures

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	5	5:2-5:3	13
6 months	4:4-4:5	8	8:3-8:5	20
1 year	4:1-4:1	9	8:3-8:5	19
2 years	5:4-5:5	9	11:3-11:5	19
3 years	NA			
4 years	14:0-14:5	20	13:6-13:1	22
5 years	≥19	20	15:0-15:5	22

BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

His BOT-2 baseline scaled scores were well below normal for the Running Speed and Agility subset, and within 1 standard deviation (SD) of normal for the Strength subset. During treatment, his performance score increased significantly for running and strength, indicating skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. Also, he showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (141%, 114% predicted [right, left]) and extensors (151%, 157%) and hip abductors (123%, 108%) and flexors (77%, 77%) as measured by HHD. The 6MWT increased from 360 meters (68% predicted) at baseline to 697 meters (105% predicted) at last assessment.

Patient 3 Radiographic Improvement in the Left Knee and Left Wrist During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+1.67	+1.00	+1.67	+1.67
RSS	0.5	0.0	0	0.5	0.5	0.0

He did not experience any TEAEs that resulted in a change in dosing regimen. Beginning on Day 4, through last assessment, he had recurrent ISRs, including erythema, pruritus, pain, discoloration, and lipohypertrophy. All were assessed by the investigator as mild and definitely related to study drug. No ectopic calcifications were found. He did not have any TEAEs assessed as both related to study drug and moderate or severe. Asfotase alfa antibodies were detected from 1 year through last assessment, with titers ranging from 64 to 512. He was positive for neutralizing antibodies only at 4.5 years of treatment.

Now, 11 years old and after 5 years of therapy, he participates in a competitive youth baseball league. He was voted his team's Most Valuable Player twice, and elected to the league's All Star Team, with a batting average of 0.740. He also plays soccer and won 5th in his triathlon group. A linear fracture through his left medial malleolus and growth plate healed within 1 month.

PATIENT 4

Patient 4 was an 8 year and 8 month-old boy at study entry. His gross motor function was delayed. At age 22 months, he had a painful cry when clothing was changed, was experiencing dolichocephaly accompanied by frontal bossing, and showed loss of height velocity. Hypophosphatasemia was found and he was diagnosed with childhood HPP. At 2 years of age, he had deciduous tooth loss and was found to have cranial pan-suture closure. Craniosynostosis repair occurred at age 3 years. Independent walking was delayed until age 3 years. Just prior to enrolling in the study, he suffered pain in his legs and ankles during activity, was slower than his classmates, and on the periphery of peer groups due to fear of falling. He was unable to hop or jump. He had adaptive physical education. He tired walking across the parking lot to enter a store, and rode in the grocery cart, although unable to climb into it. He had knock knees; metaphyseal flaring at his wrists, knees, and ankles; an unusual gait; an asymmetrical face from surgical repair of craniosynostosis; and mild rachitic chest deformity. Height and weight Z-scores were both low (-2.9 and -2.5, respectively). Baseline serum levels were ALP (29 U/L), PPi (4.4 μM), PLP (218 ng/mL), 25-hydroxyvitamin D (75 pmol/mL), PTH (1.3 pmol/L), and Ca^{2+} (2.5 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 86 U/L. Normal reference ranges of the other parameters are also presented in Table 1.

He initiated asfotase alfa therapy at 6 mg/kg/week given as three injections per week. Figure 4 shows the radiographic improvement in his left wrist and knee during treatment. At 1.5 months of treatment, he rode his bike 3 times longer than previously, started to hop and jump, climbed into a grocery cart, and could run. He now walks through airports without tiring. By 3 months of treatment, he no longer had pain with activities.

His baseline BOT-2 scaled scores were well below normal for the Running Speed and Agility subset and the Strength subset. With treatment, his performance scores increased significantly for running and strength. He showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (95%,100% predicted [right, left]) and extensors (127%, 112%) and hip abductors (79%, 99%) and flexors (64%, 66%) as measured by HHD. The 6MWT increased from 300 meters (51% of predicted) at baseline to 560 meters (83% of predicted) at last assessment.

Patient 4 Functional Outcome Measures (BOT-2)

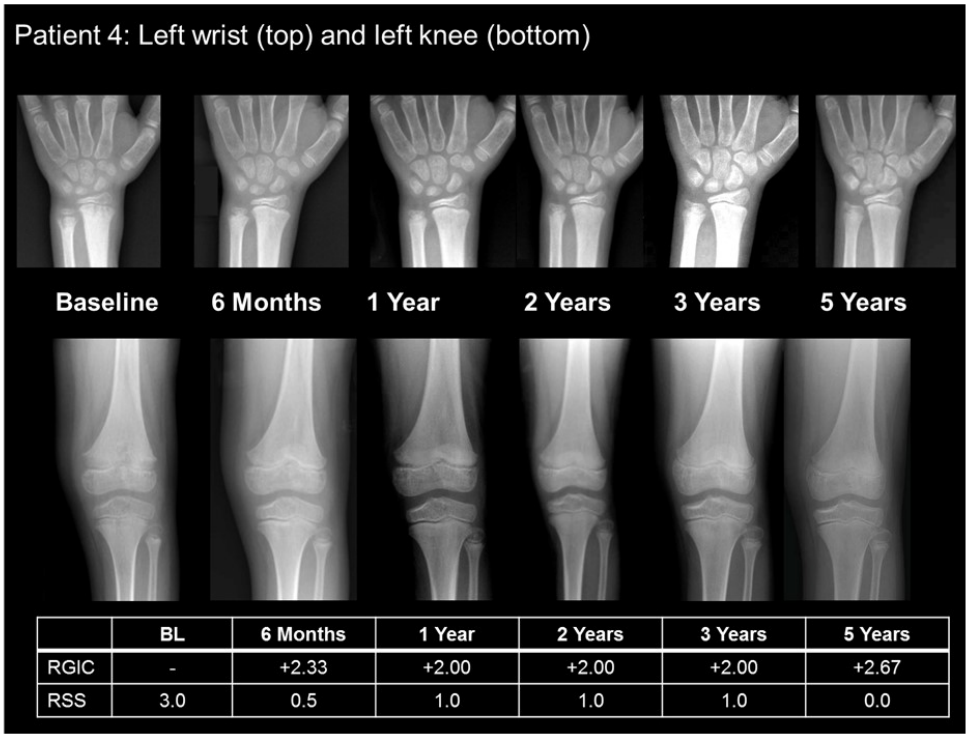
Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	4	6:6-6:8	10
6 months	4:1-4:1	6	8:0-8:2	12
1 year	6:0-6:2	8	9:3-9:5	14
2 years	6:3-6:5	8	12:0-12:5	17
3 years	9:0-9:2	12	13:0-13:5	19
4 years	13:0-13:5	16	14:0-14:5	18
5 years	10:3-10:5	13	13:6-13:1	17

BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

He did not experience TEAEs that resulted in a change of dosing regimen. Beginning on Day 8 through last assessment, he had recurrent ISRs, including erythema, pruritus, pain, discoloration, and lipohypertrophy. All were assessed by the investigator as mild and probably or definitely related to study drug. Two events of ectopic calcification were reported: conjunctival deposits on temporal and nasal conjunctiva at 3 years of treatment. The events were assessed by the investigator as mild and probably related to study drug and are ongoing. At the first event, quantitation of plasma PPi (2.3 μM), serum Ca^{+2} (2.4 mmol/L), and urinary calcium:creatinine (0.2 mmol/mmol) were normal and no changes were made in dosing regimen. He was negative for anti-asfotase alfa antibodies at baseline, but positive results began at 1.5 months and continued. He was positive (values >4.5%) for neutralizing antibodies only at 4 years of treatment.

Presently, at age 14 years and with 5 years of asfotase alfa therapy, he is a healthy teenager. He plays flag football, pick-up basketball, and has no physical education restrictions. His height has improved to Z=-2.0.

Patient 4 Radiographic Improvements in the Left Knee and Wrist During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.33	+2.00	+2.00	+2.00	+2.67
RSS	3.0	0.5	1.0	1.0	1.0	0.0

PATIENT 5

Patient 5, 12 years and 0 months-old at study entry, developed failure to thrive at 1 year of age and had delayed gross motor skills at the time of onset of walking at age 21 months. A muscle biopsy obtained at age 3 years was normal. Childhood HPP was diagnosed at age 3.5 years. Premature tooth loss began at age 2 years, and by age 5 years, he had lost 7 teeth. He had knock knees, an unusual gait, could not keep up with peers, and did not participate in physical education. He was on the swim team, but always finished at least a pool length behind. For safety, he dove from the pool side, rather than the starting block. He had difficulty with inclines and steps. Ectopic calcification in the left eye was noted prior to study enrollment. At study entry, his height was Z-score=-1.3 and weight Z-score=-0.1. Serum levels were ALP (35 U/L), PPi (4.2 µM), PLP (267 ng/mL), 25-hydroxyvitamin D (57 pmol/mL), PTH (2.7 pmol/L), and Ca^{2+} (2.4 mmol/L). Normal reference ranges are presented in Table 1. The age- and sex-adjusted lower limit of the reference range for ALP was 95 U/L. The reference ranges of the other parameters are also presented in Table 1.

He initiated asfotase alfa therapy at 9 mg/kg/week given as 3 injections. He had erythematous ISRs that necessitated a dose reduction to 6 mg/kg/week at month 3.5 of treatment. For the extension study, per protocol, his dose was decreased to 3 mg/kg/week. However, 3 weeks later he noticed returning fatigue and weakness with inability to ride his bicycle uphill or climb steps with alternating feet and had a return of pain with activities. Then, 3 weeks later with treatment, his parents noticed return of his unusual gait. Accordingly, 3 months after the dose reduction, his dose was increased to 6 mg/kg/week given as 6 injections. The drug concentration was decreased in an attempt to decrease the ISRs.

Patient 5 Functional Outcome Measures (BOT-2)

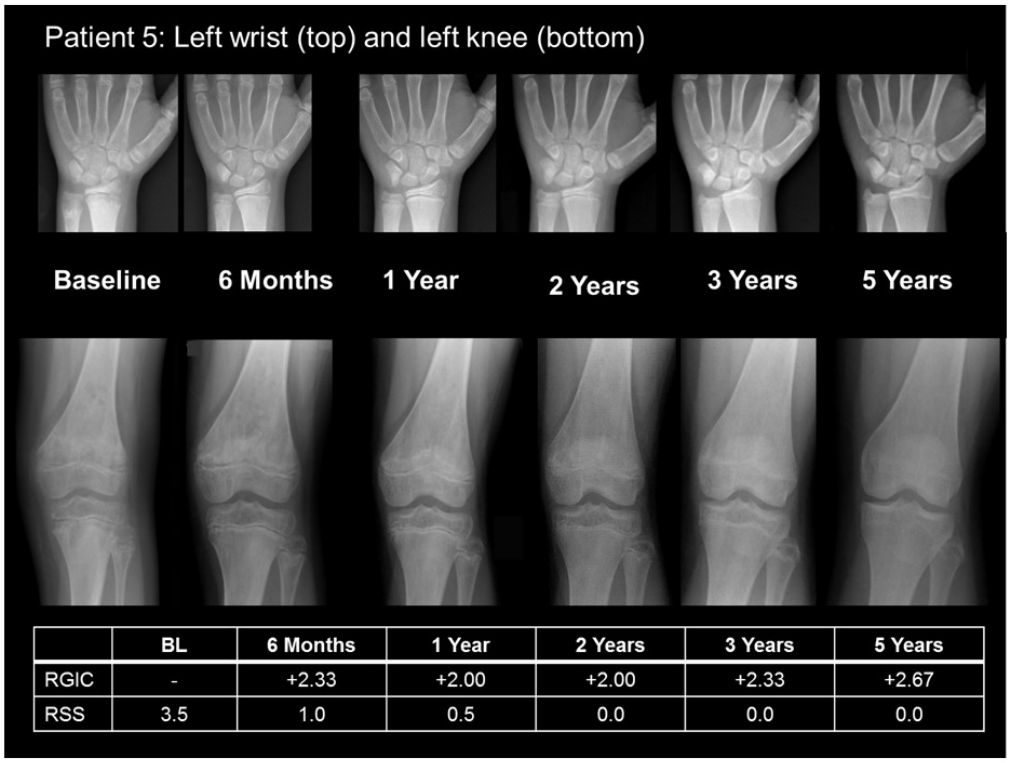
Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	1	4:2-4:3	3
6 months	5:0-5:1	5	7:9-7:1	9
1 year	5:2-5:3	5	6:6-6:8	7
2 years	7:9-7:11	8	10:6-10:8	11
3 years	19 and above	19	12:6-12:1	12
4 years	8:6-8:8	9	13:0-13:5	13
5 years	8:6-8:8	9	12:6-12:1	12
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available. [a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).				

The baseline BOT-2 score was well below normal for the Running Speed and Agility subset and the Strength subset. During treatment, his performance scores increased for running and strength. He showed some increase over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (73%, 85% predicted [right, left]) and extensors (44%, 47%) and hip abductors (91%, 101%) and flexors (34%, 35%), as measured by HHD. This was also demonstrated by his ability to walk farther (6MWT) during treatment, increasing from 301 meters (45% predicted) at baseline to 580 meters (82% predicted) at last assessment.

He had recurrent ISRs, including erythema, pruritus, pain, discoloration, and hypertrophy, beginning Day 4 and to last assessment. All were considered by the investigator as mild or moderate and definitely related to the study drug. Because of these ISRs, 2 dose reductions occurred during the time frame of the ISRs: 4.5 mg/kg/week (1.5 mg/kg/day × 3/week) for 1 week and 6 mg/kg/week (2 mg/kg/day × 3/week) for 9 weeks. No IARs were reported. Additional ophthalmological ectopic calcification was noted at Month 10 of treatment and was ongoing at last assessment. It was considered mild and possibly related to study drug. At the time of the calcification, plasma PPi (2.3 μM), serum Ca^{2+} (2.5 mmol/L), and urinary calcium:creatinine (0.6 mmol/mmol) levels were within normal limits. The dose was not changed in response to this event.

He started to ride a school bus and stopped using the elevator at school after 2 years of therapy. His swim time decreased by 26 seconds, and he won a race. Presently, at 17 years of age and with 5 years of therapy, he climbs trees, hikes in the mountains, and rides a motorcycle.

Patient 5 Radiographic Improvement in the Left Knee and Left Wrist During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.33	+2.00	+2.00	+2.33	+2.67
RSS	3.5	1.0	0.5	0.0	0.0	0.0

PATIENT 6

This 12 year, 5 month-old boy at study entry presented with sagittal suture closure at age 5 months. Craniosynostosis repair with calvarial morselization occurred at age 7 months. At the age 18 months, he had decreased visual acuity. At 2 years of age, the morselized bone was removed, and he had optic nerve decompression. However, he was declared legally blind. Between 2-3 years of age, he had seizures from increased intracranial pressure. A Chiari I malformation was noted at age 9 years. In addition, he had premature tooth loss at age 13 months. By age 2 years, he had lost 6 teeth. He walked independently at age 18 months and had knock knees. At baseline, he suffered pain in his arms, legs, and ankles that occurred after walking 2 blocks. Due to fatigue, he had to use arm floats and swim with an adult walking beside him. At study entry, his height was Z-score=-1.1 and weight Z-score=-1.0. Baseline serum levels were ALP (37 U/L), PPi (4.0 µM), PLP (151 ng/mL), 25-hydroxyvitamin D (76 pmol/mL), PTH (4.0 pmol/L), and Ca^{2+} (2.4 mmol/L). Normal reference ranges are presented in Table 1. The age- and sex-adjusted lower limit of the reference range for ALP was 95 U/L.

He initiated asfotase alfa treatment at a weekly dose of 6.0 mg/kg SC (3 injections/week). Upon entry into the extension study, his dose was decreased per protocol to 3.0 mg/kg/week. At Week 52, the patient began receiving 6.0 mg/kg/week as part of a study-wide dose increase. At Week 55, he incorrectly received a weekly dose of just 3.0 mg/kg SC instead of the 6.0 mg/kg/week dose, but the dose was corrected by the end of that study week.

Patient 6 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	5:8-5:9	6	6:3-6:5	7
6 months	7:0-7:2	8	7:6-7:8	8
1 year	7:6-7:8	8	7:9-7:1	9
2 years	6:3-6:5	6	8:6-8:8	9
3 years	NA	NA	NA	NA
4 years	8:6-8:8	9	7:9-7:1	7
5 years	8:0-8:2	8	7:9-7:1	7

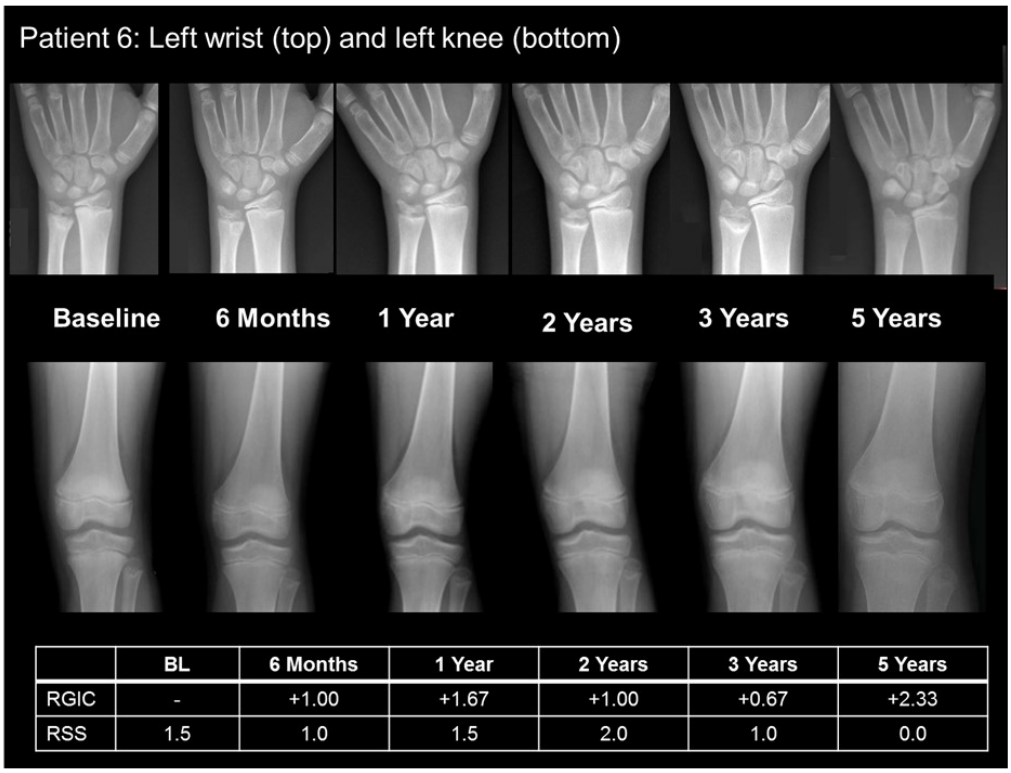
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

Despite his visual impairment, patient 6 could participate in some of the functional assessments. His BOT-2 scores were well below normal for the Running Speed and Agility subset and the Strength subset. With treatment, his performance scores increased for running and strength, indicating skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. Also, he showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (92%, 79% predicted [right, left]) and extensors (39%, 32%) and hip abductors (91%, 85%) and flexors (37%, 39%), as measured by HHD. The 6MWT increased from 456 meters (68% predicted) at baseline to 568 meters (80% predicted) at last assessment. His Z-scores for height (-1.1 to +0.2) and weight (-1.0 to +0.5) improved with treatment.

He did not experience any TEAE that resulted in a change in dosing regimen. Recurrent ISRs included erythema, swelling, discoloration, and lipohypertrophy, beginning Day 5 up to last assessment. All were considered by the investigator as mild or moderate and probably or definitely related to study drug. Most were ongoing at last assessment. One ectopic calcification occurrence was reported, a moderate conjunctival deposit found at 5 years of treatment. The event was considered probably related to study drug. At baseline, he was negative for anti-asfotase alfa antibodies, but positive results began at Year 1 of treatment and then persisted. He was not positive for neutralizing antibody activity at any timepoint.

At 1.5 months of therapy, he shoveled snow with no pain. By 4.5 months of therapy, he was able to swim laps without the arm floats or "supervisor," as his strength and endurance had improved. Now, at age 18, he is an active young man who attends public high school and a school for the blind. He is able to march in the marching band. His visual acuity did not seem to improve from treatment. At 6 months of treatment, he developed abdominal lipohypertrophy that had increased in size at Month 30. This was because he continued to inject this one site as it hurt less. Now this is cosmetically problematic. Presently, his height is Z=+0.2 and weight is Z=+0.5.

Patient 6 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+1.00	+1.67	+1.00	+0.67	+2.33
RSS	1.5	1.0	1.5	2.0	1.0	0.0

PATIENT 7

This 6 year, 4 month-old boy at study entry presented with tooth loss at age 14 months. Walking began at age 13 months and he developed a knock knee stance. At age 18 months, he had metaphyseal flaring at the wrists. Radiographs showed rickets, but the diagnosis of childhood HPP was not made until biochemical studies were performed at age 2.5 years. By age 5 years, 11 teeth were lost. Pain occurred in his wrists, knees, and ankles and limited his activities. He climbed stairs by pulling himself up using both hands on the railing. In school, he walked with an aide and did not participate in physical education. His parents were considering making the house handicap-accessible. At baseline, his height was Z-score=-0.9 and weight Z-score=-1.3. Serum levels were ALP (48 U/L), PPi (5.6 µM), PLP (>100 ng/mL), 25-hydroxyvitamin D (62 pmol/mL), PTH (pmol/L), and Ca^{2+} (2.5 mmol/L). Normal reference ranges are presented in Table 1. He initiated treatment at a weekly dose of 9.0 mg/kg SC (3 injections/week). The age- and sex-adjusted lower limit of the reference range for ALP was 93 U/L.

On Day 13 of treatment, he climbed stairs with reciprocating steps. Friends, teachers, and family commented on his increased activity and fearless approach to activities.

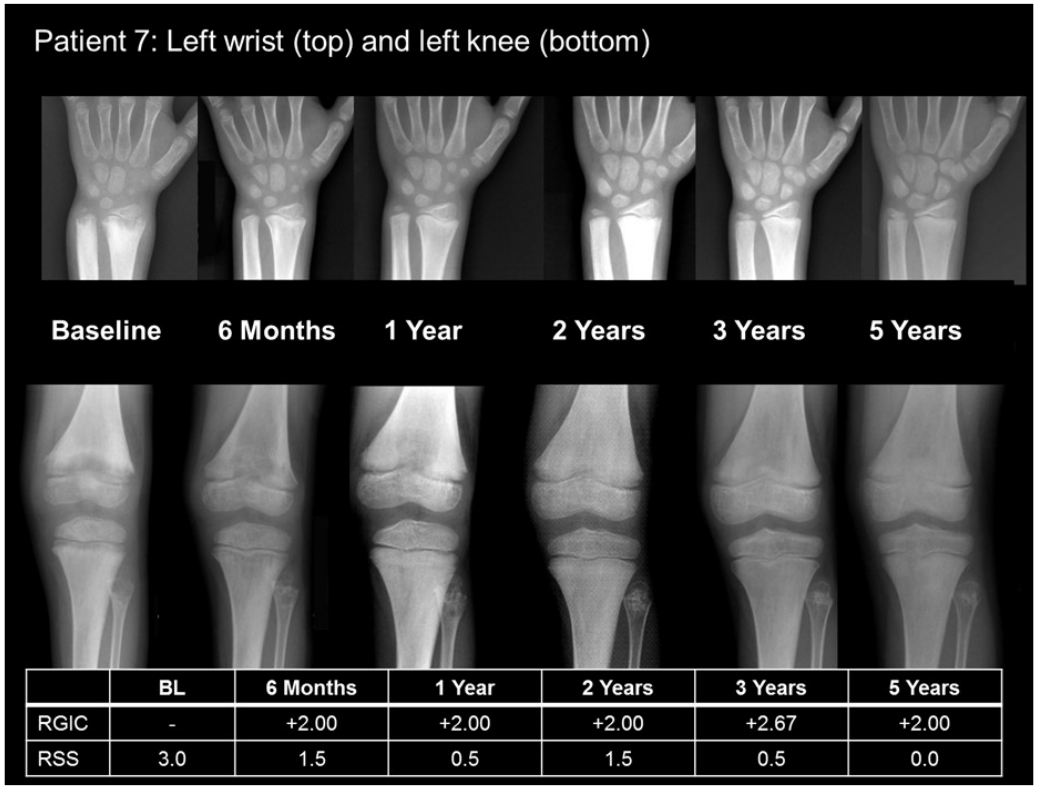
Patient 7 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	4	Below 4	4
6 months	4:6-4:7	8	6:0-6:2	13
1 year	4:8-4:9	7	7:3-7:5	15
2 years	4:1-4:1	7	8:9-9:1	18
3 years	7:0-7:2	10	9:3-9:5	22
4 years	NA	NA	NA	NA
5 years	11:0-11:2	16	13:0-13:5	19

BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

The baseline BOT-2 score was below normal for the Running Speed and Agility (scaled) subset, and below normal for the Strength subset. During treatment, his performance scores increased for running and strength. He also demonstrated improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. He showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (126%, 133% predicted [right, left]) and extensors (131%, 130%) and hip abductors (112%, 120%) and flexors (126%, 133%), as measured by HHD. The 6MWT increased from 308 meters (56% predicted) at baseline to 572 meters (87% predicted) at last assessment. His Z-scores for height (-0.9 to -0.4) and weight (-1.3 to -0.1) also improved during treatment.

Patient 7 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+2.00	+2.00	+2.67	+2.00
RSS	3.0	1.5	0.5	1.5	0.5	0.0

He did not experience any TEAEs that resulted in a change in dosing regimen. Starting on Day 8 of treatment and continuing throughout the study, he had recurrent ISRs, including erythema, discoloration, and atrophy. All were assessed by the investigator as mild and considered probably or definitely related to study drug. No IARs or ectopic calcifications were reported. He was negative for anti-asfotase alfa antibodies at baseline, but

positive beginning at 1.5 months of treatment and continuing throughout the study. He was not positive for neutralizing antibody activity at any timepoint.

Now 11 years old and after 5 years of therapy, he is a healthy boy who plays intramural baseball, attends Boy Scout camp, is faster than his peers, and does not remember his prior limitations.

PATIENT 8

This 6 year, 0 month-old girl began losing teeth at age 19 months and lost 6 teeth by age 5 years. At age 5.5 years, she wore a mandibular partial plate. She walked at age 20 months, but was slower than her younger sisters, and her 2-legged hop and run differed from her sisters'; on family hikes, she wore out and was carried on her father's shoulders. Three months prior to study entry, a new dentist recommended her first radiographs that showed metaphyseal changes. At age 5.5 years, her diagnosis was changed from odontohypophosphatasia to childhood HPP. At baseline, her height was Z-score=-1.0 and weight Z-score=-0.9. Serum levels were ALP (65 U/L), PPi (4.1 μM), PLP (156 ng/mL), 25-hydroxyvitamin D (49 pmol/mL), PTH (3.9 pmol/L), and Ca^{2+} (2.4 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 96 U/L. The reference ranges of the other parameters are also presented in Table 1.

She initiated treatment at a weekly dose of 9.0 mg/kg SC (3 injections/week).

Patient 8 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	5	4:4-4:5	7
6 months	4:8-4:9	8	5:6-5:7	12
1 year	5:0-5:1	8	10:0-10:2	21
2 years	6:0-6:2	10	9:6-9:8	17
3 years	NA	NA	NA	NA
4 years	10:0-10:2	16	10:6-10:8	17
5 years	8:0-8:2	13	11:6-11:8	17

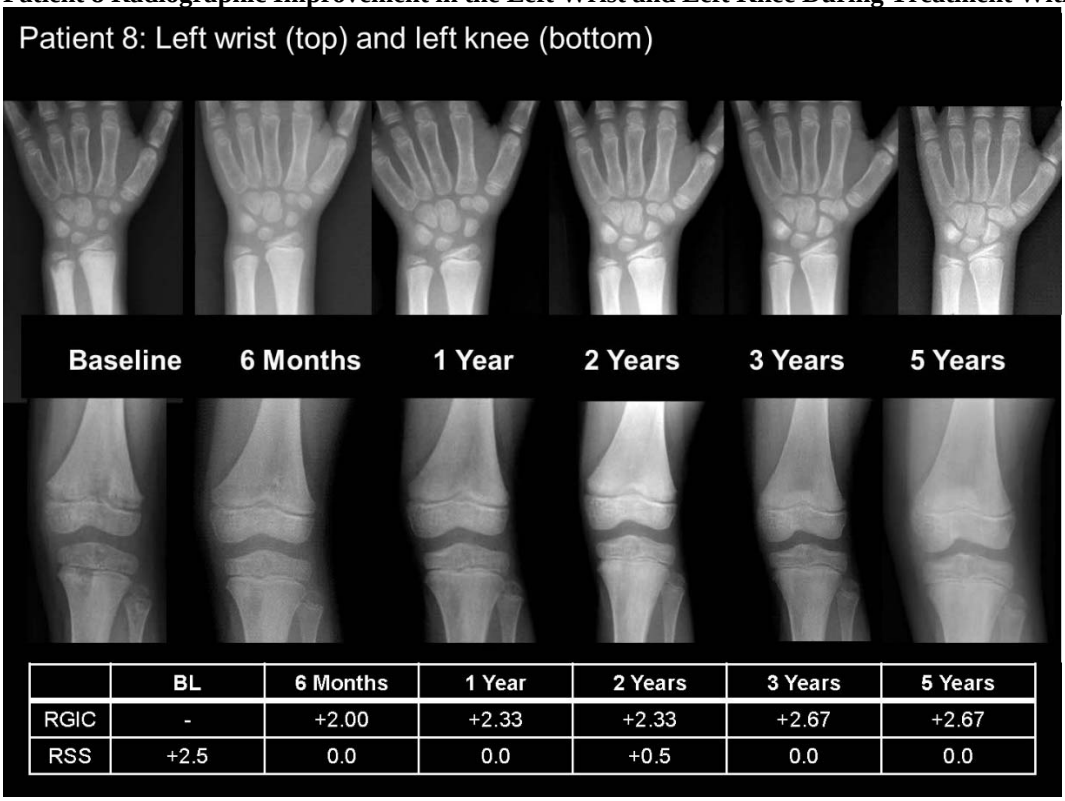
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

At 1.5 months of therapy, she started to skip and was able to ascend stairs with reciprocal steps. The baseline BOT-2 was well below normal for the Running Speed and Agility subset and the Strength subset. With treatment, her performance scores increased for running and strength, indicating skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. She showed significant increases over 5 years of treatment in torque and median force for individual muscle groups including knee flexors (97%, 115% predicted [right, left]) and extensors (138%, 147%) and hip abductors (66%, 85%) and flexors (58%, 52%), as measured by HHD. The 6MWT increased from 423 meters (80% predicted) at baseline to 538 meters (83% predicted) at last assessment. Her Z-scores improved for height (-1.0 to -0.3) and weight (-0.9 to -0.2) within normal range.

She did not experience any TEAEs that resulted in a change in dosing regimen. She had multiple ISRs that included discoloration, erythema, and lipohypertrophy; the events were assessed as mild and definitely or probably related to study drug. No IARs were reported. Conjunctival Ca^{2+} deposits were observed bilaterally at 2 years of treatment, and persisted. No papilledema was reported. The conjunctival Ca^{2+} deposits were mild and assessed as possibly related to study drug. She became positive for anti-asfotase alfa antibodies at 3 months onward, but was not positive for neutralizing antibody activity at any timepoint.

Now, at age 11 years and after 5 years of therapy, she is able to keep up with peers and participates in physical education.

Patient 8 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+2.33	+2.33	+2.67	+2.67
RSS	+2.5	0.0	0.0	+0.5	0.0	0.0

PATIENT 9

Patient 9, a 7 year and 7 month-old boy at baseline, had fractures, hypermobility, joint pain, premature loss of deciduous teeth, muscle pain and weakness, an unusual gait, and rickets as symptoms of childhood HPP. Upon study entry, he was below average in height and weight for his age, with mild disability and pain. There was also bone pain, knock knees, tooth abscesses, a prior diagnosis of "hypophosphatemic rickets," and difficulty gaining weight from feeding difficulties. He had a fractured left wrist (torus fracture) at study entry. Baseline serum levels were ALP (54 U/L), PPi (3.7 µM), PLP (>100 ng/mL), 25-hydroxyvitamin D (91 pmol/mL), PTH (2.5 pmol/L), and Ca^{2+} (2.7 mmol/L). Normal references are presented in Table 1. The age- and sex-adjusted lower limit of the reference range for ALP was 86 U/L.

He initiated asfotase alfa treatment at a weekly dose of 6.0 mg/kg SC (3 injections/week).

Patient 9 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	3	4:1-4:1	8
6 months	5:6-5:7	9	6:6-6:8	11
1 year	7:3-7:5	11	8:6-8:8	15
2 years	7:3-7:5	10	9:0-9:2	14
3 years	8:6-8:8	12	10:0-10:2	15
4 years	11:0-11:2	15	11:0-11:2	16
5 years	8:6-8:8	11	11:6-11:8	15

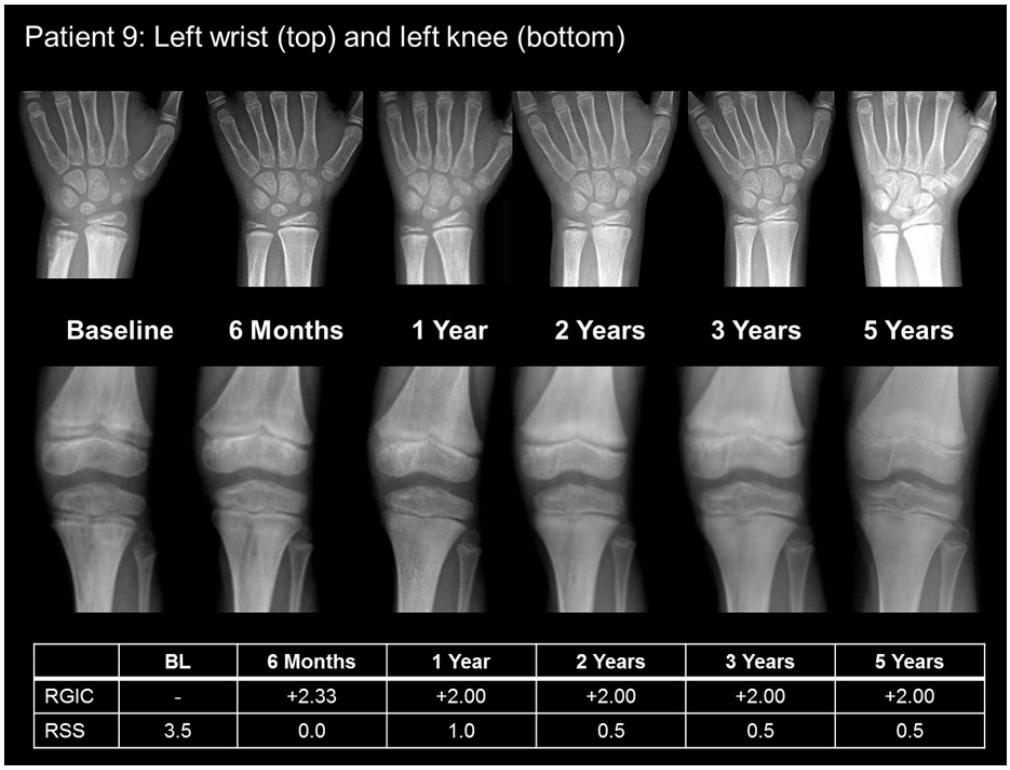
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

His baseline BOT-2 scaled scores were well below normal for the Running Speed and Agility subset and the Strength subset. During treatment, his performance scores increased for running and strength. He showed increases over 5 years of treatment in median force for individual muscle groups, including knee flexors (136%, 113% predicted [right, left]) and extensors (184%, 154%) and hip abductors (174%, 172%) and flexors (109%, 134%) as measured by HHD. The 6MWT increased from 379 meters (65% predicted) at baseline to 534 meters (79% predicted) at last assessment. His Z-scores for height (-0.6 to 0.0) and weight (-1.1 to +1.1) also improved.

He did not experience any TEAEs that resulted in a change of the dosing regimen. He had recurrent ISRs, including erythema, pruritus, pain, discoloration, swelling, nodule formation, induration, and atrophy, which continued to last assessment. All were assessed by the investigator as mild or moderate and definitely related to study drug. He also had multiple IARs that were considered mild and definitely related to the study drug. He also developed sonographic evidence of nephrocalcinosis at the lower pole of the left kidney (very subtle) (Week 12). The investigator considered this observation to not be clinically significant. Follow-up ultrasounds studies were normal, with no renal calculi identified at any time. He was negative for anti-asfotase alfa antibodies at baseline, but tested positive at most study visits starting at the 2-year study visit; he was not positive for neutralizing antibody activity at any timepoint.

Now, 12 years of age and after 5 years of treatment, his overall health and ability to participate in both normal daily activities as well as more vigorous activities are approaching those of healthy children.

Patient 9 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.33	+2.00	+2.00	+2.00	+2.00
RSS	3.5	0.0	1.0	0.5	0.5	0.5

PATIENT 10

Patient 10 was a 7 year, 2 month-old boy at baseline whose first signs and symptoms of HPP included difficulty gaining weight (small for gestational age), failure to thrive, hypercalcemia, hypercalciuria, and nephrocalcinosis (beginning at 1 month of age). His medical history included an abnormally shaped head and chest, bone pain, rickets, delayed walking, unusual gait, non-healing fractures and pseudofractures, feeding difficulties,

excessive cavities and premature tooth loss, muscle pain and weakness, joint pain and swelling, knock knees, thickening of the wrists and knees, metaphyseal lesions and lucencies, gross motor and speech delays, persisting hypercalcemia, and hypercalciuria. Upon study entry, he was well below average in height and weight, with moderate disability. His baseline pain was severe and he required daily morphine. He was wheelchair-dependent for any travel distance. Baseline serum levels were ALP (32 U/L), PPi (5.1 μM), PLP (233 ng/mL), 25-hydroxyvitamin D (63 pmol/mL), PTH (3.8 pmol/L), and Ca^{2+} (2.5 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 86 U/L. The reference ranges of the other parameters are presented in Table 1.

He initiated treatment at a weekly dose of 6.0 mg/kg SC (3 injections/week).

Patient 10 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	3	Below 4	2
6 months	Below 4	4	Below 4	4
1 year	4:4-4:5	5	Below 4	3
2 years	4:4-4:5	4	4:4-4:5	5
3 years	5:6-5:7	7	8:9-8:1	12
4 years	6:3-6:5	8	10:6-10:8	14
5 years	7:0-7:2	8	9:9-9:1	12

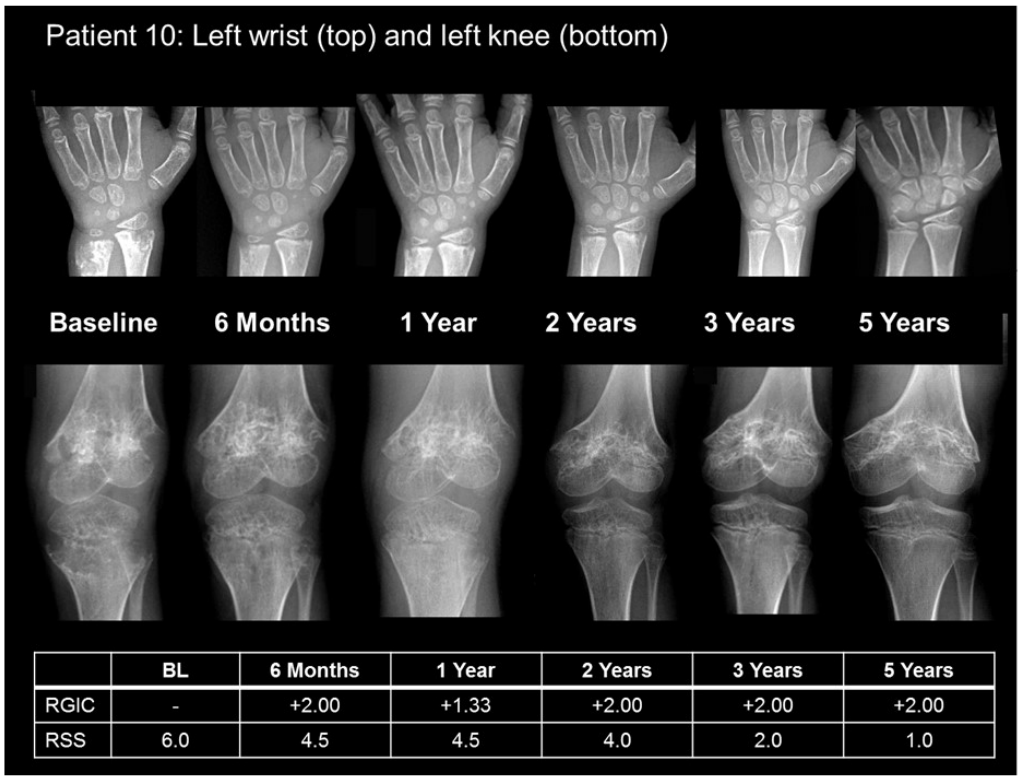
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

His baseline scaled scores were well below normal for the Running Speed and Agility subset and the Strength subset. During treatment, his performance scores increased significantly for running and strength, indicating skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. He showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (52%, 61% predicted [right, left]) and extensors (not measured, 76%) and hip abductors (94%, 85%) and flexors (52%, 50%), as measured by HHD. The 6MWT increased from 217 meters (41% predicted) at baseline to 520 meters (84% predicted) at last assessment. His Z-scores for height (-6.6 to -5.8) and weight (-8.2 to -5.4) improved.

He did not experience any TEAEs that resulted in a change to dosing regimen. He was reported to have 2 ISRs: mild discoloration and atrophy of the skin injection sites. They were assessed as definitely related to study drug and were ongoing at last assessment. After 2 years of treatment, moderate growth hormone deficiency was noted during repeated endocrine challenges. Consequently, growth hormone treatment was initiated and continued throughout the study. At baseline, a renal ultrasound showed a 5-mm simple cyst in the right kidney, considered by the investigator not to be clinically significant and unrelated to HPP. At 6 months of treatment, renal ultrasound revealed a small (3-mm) cyst in the left kidney. Additional ultrasounds indicated the cysts in both kidneys remained unchanged as of the last visit. No renal calculi were identified at any time. He was negative for anti-asfotase alfa antibodies at baseline and throughout most of the study except at 1 and 2 years. He was negative for neutralizing antibody activity at any timepoint.

Now, age 12 years and after 5 years of treatment, his overall health and ability to participate in both normal daily activities as well as more vigorous activities is improved. He no longer requires pain medication, is fully ambulant, and participates in all sports, including skating.

Patient 10 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+1.33	+2.00	+2.00	+2.00
RSS	6.0	4.5	4.5	4.0	2.0	1.0

PATIENT 11

Patient 11, a 10 year and 11 month-old boy at study entry for whom initial studies did not reveal a TNSALP gene mutation, had his first signs and symptoms of hypophosphatasia at approximately age 1 month, including joint pain and swelling, hypercalcemia, and hyperphosphatemia. He was below normal in height and weight, with disability and pain. There was bilateral bowing in his arms, bone pain severe enough to require pain medication and limit activity, dental abscesses and excessive cavities, premature tooth loss, delayed speech and walking, muscular weakness, knock knees, unusual gait or way of walking or running, abnormally shaped chest, seizures, difficulty eating/swallowing, joint pain and swelling, hyperextensible joints, gross motor delay, hypercalcemia, and hypercalciuria. There was no fracture history. Baseline serum levels were ALP (27 U/L), PPi (5.5 μM), PLP (297 ng/mL), 25-hydroxyvitamin D (74 pmol/mL), PTH (6.8 pmol/L), and Ca^{2+} (2.5 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 95 U/L. The reference ranges of the other parameters are also presented in Table 1.

He initiated asfotase alfa treatment at a weekly dose of 9.0 mg/kg SC (3 injections/week).

Patient 11 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	2	Below 4	2
6 months	5:2-5:3	6	6:9-6:1	8
1 year	5:2-5:3	5	7:6-7:8	8
2 years	8:0-8:2	9	8:6-8:8	10
3 years	7:9-7:1	8	8:6-8:8	10
4 years	9:6-9:8	11	9:0-9:2	10
5 years	13:0-13:5	14	11:6-11:8	11

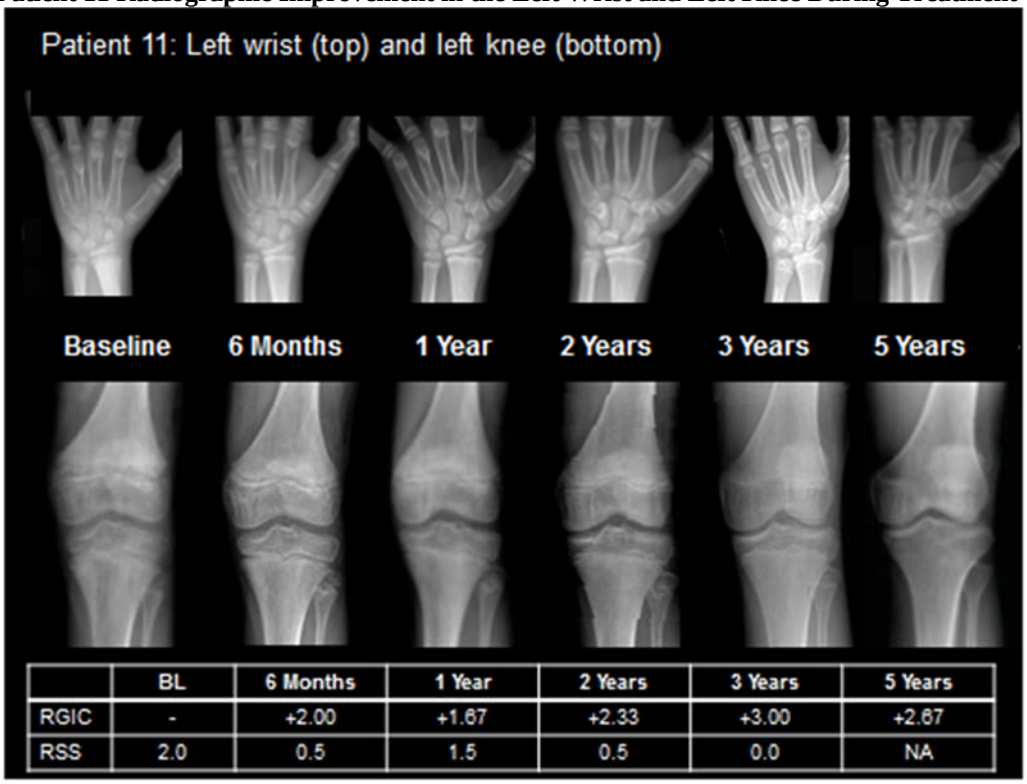
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available.
[a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).

His baseline scaled scores were well below normal for the Running Speed and Agility subset and the Strength subset. During treatment, his performance scores increased for running and strength, indicating skill acquisition and an improved ability to run and pick up an object and to control acceleration and deceleration during ambulation. He showed significant increases over 5 years of treatment in torque and median force for individual muscle groups, including knee flexors (153%, 135% predicted [right, left]) and extensors (148%, 124%) and hip abductors (166%, 152%) and flexors (103%, 99%), as measured by HHD. The 6MWT increased from 430 meters (66% predicted) at baseline to 651 meters (92% predicted) at last assessment. His Z-scores for height (-1.3 to -0.7) and weight (-1.3 to -0.5) were similar over treatment.

He did not experience any TEAEs that changed his dosing regimen. He had recurrent ISRs, also considered IARs, including pain on Day 8, pruritus on Day 10, both lasting 1 day, and erythema, which began on Day 10 and resolved on Day 26. Other ISRs that occurred later in the study and were not considered IARs were discoloration, atrophy, and erythema. Sonography detected a 1 cm renal calculus in the upper pole right kidney and was reported as a mild TEAE unrelated to study drug. Renal ultrasound was normal at 3 years. A new stone in the right kidney was noted at last assessment. He was negative for anti-asfotase alfa antibodies at baseline. Antibodies were detected at 1.5 months of treatment and all other study visits, with titers increasing from 4 at 1.5 months to 512 at 1.5 years, and then decreasing to 64 at the last assessment. He was positive for neutralizing antibody activity.

Now, 15 years old and after 5 years of treatment, his overall health, stamina, and ability to participate in both normal daily activities as well as more vigorous activities are greatly improved.

Patient 11 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+1.67	+2.33	+3.00	+2.67
RSS	2.0	0.5	1.5	0.5	0.0	NA

PATIENT 12

This boy was 10 years, 5 months old at study entry and diagnosed with childhood HPP at 9 years of age. At age 17 months, he had delayed walking, a waddling gait, pain in his femurs, legs, and feet that limited activities, a modified Gowers' maneuver, and abnormal radiographs. However, evaluations did not yield a diagnosis although HPP had been considered at about age 3 years. He had premature tooth loss with root intact beginning at age 3 years and lost 4 teeth by age 5 years. His easy fatigue, lack of participation in activities, and difficulty climbing steps in

and out of a minivan had been attributed to his Asperger syndrome. Prior to enrolling in the study, he sat on a bench while his family shopped. He needed assistance climbing steps. He was home-schooled, and although he liked the pool, he could not swim. He was heavy with weight Z-score=+2.3 and height Z-score=-0.0 at study entry. Baseline serum levels were ALP (49 U/L), PPi (4.9 μM), PLP (84 ng/mL), 25-hydroxyvitamin D (54 pmol/mL), PTH (1.9 pmol/L), and Ca^{2+} (2.5 mmol/L). The age- and sex-adjusted lower limit of the reference range for ALP was 95 U/L. The reference ranges of the other parameters are also presented in Table 1.

He initiated treatment at a weekly dose of 9.0 mg/kg SC (3 injections/week), reduced to 6.0 mg/kg at Week 6 following detection of low plasma PLP. He now receives 80 mg of asfotase alfa 6x/week even though his body weight is 113 kg.

Patient 12 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	2	Below 4	1
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available. [a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).				

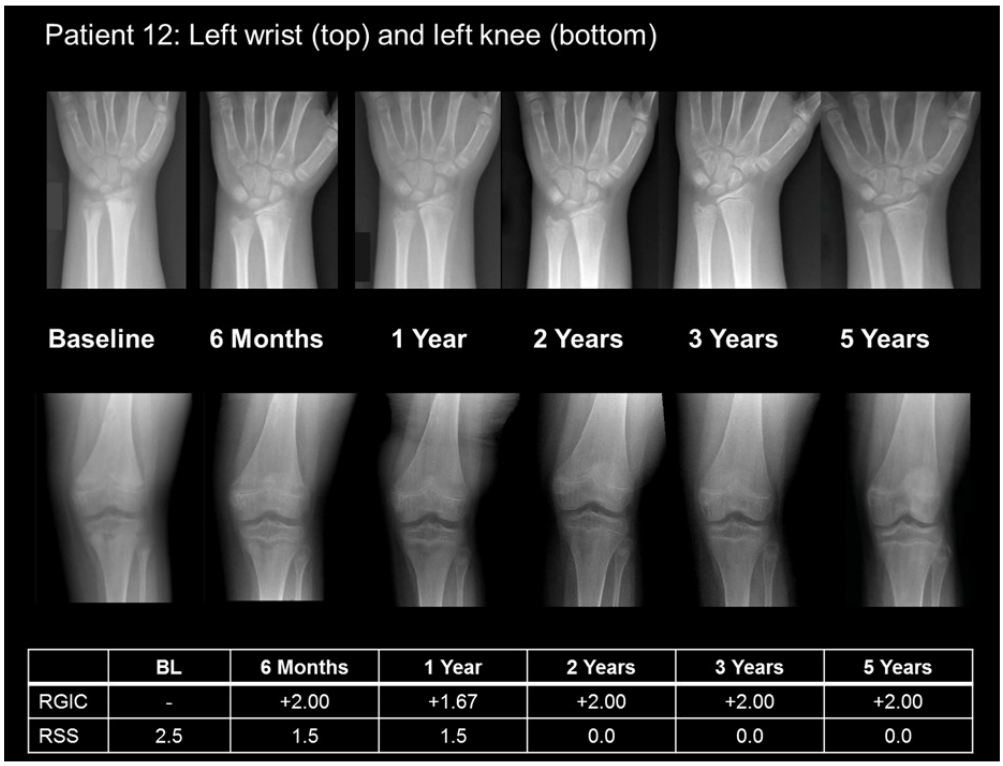
Due to Asperger syndrome, he was unable to complete physical therapy assessments because he could not fully comply with instructions to provide best effort. Therefore, his BOT-2 scaled scores were only available for baseline. They demonstrated significant delay. His height Z-score (-0.0 to -0.5) declined during the study, while weight Z-score (2.3 to 2.2) remained elevated.

He did not experience any TEAEs during treatment that warranted a regimen change. Starting on Day 2 of treatment, and then continuing, he had recurrent ISRs including erythema, pain, swelling, discoloration, and lipohypertrophy. All were assessed by the investigator as mild or moderate and definitely related to study drug. Renal ultrasound revealed small kidney stones at 4 years of treatment, but no evidence of medullary nephrocalcinosis persisting through last assessment. The event was reported as a TEAE (preferred term: nephrolithiasis), assessed by the investigator as mild and possibly related to study drug. At 1.5 months, a TEAE of low PLP (0.9 ng/mL) was recorded.

He was negative for asfotase alfa antibodies at baseline. Positive results were observed beginning at 1.5 months of treatment and continuing through 2 years (with the exception of 1.5 years). Negative results were observed at 2.5 years through the last assessment. He was positive (value >4.5%) for neutralizing antibody activity from Months 3 to 11, then negative thereafter.

Currently, at 15 years of age and after 5 years of asfotase alfa therapy, he participates in more activities. He swims easily, hikes, and attends a private school. He recently helped carry boxes to a 3rd-floor walk-up apartment.

Patient 12 Radiographic Improvement in the Left Wrist and Left Knee During Treatment With Asfotase Alfa



	BL	6 Months	1 Year	2 Years	3 Years	5 Years
RGIC	-	+2.00	+1.67	+2.00	+2.00	+2.00
RSS	2.5	1.5	1.5	0.0	0.0	0.0

PATIENT 13

Patient 13, a 10 year and 0 months-old boy at baseline, manifested his first symptoms of HPP at age 3 months, including delayed walking, feeding difficulties, and failure to thrive. He was subsequently diagnosed with infantile HPP. Upon study entry, he was below average in height and weight, had mild difficulty in functional activities, and had mild pain. His medical history was significant for an abnormally shaped chest and head, rickets, unusual gait, hypermobility, difficulty eating and swallowing, premature tooth loss, and suspected bilateral nephrocalcinosis. There was no history of fractures. He had surgically corrected craniosynostosis and insertion of a gastrostomy tube prior to entering the study. Serum levels were ALP (58 U/L), PPi (6.5 µM), PLP (245 ng/mL), 25-hydroxyvitamin D (78 pmol/mL), PTH (2.1 pmol/L), and Ca^{2+} (2.4 mmol/L). Normal reference values are presented in Table 1. The age- and sex-adjusted lower limit of the reference range for ALP was 95 U/L.

He initiated asfotase alfa treatment at a weekly dose of 9.0 mg/kg SC (3 injections/week). He continued this regimen until electing discontinuation for elective spinal surgery for pre-existing scoliosis. He had received treatment for only 1 month.

At baseline, he demonstrated radiographic evidence of mild rickets, as indicated by an RSS score of 3.0. The RGI-C score was not utilized because of missing post-baseline images.

Patient 13 Functional Outcome Measures (BOT-2)

Visit	Running Speed and Agility Age-equivalent Score (yrs:mos)	Scaled Score[a]	Strength Age-equivalent Score (yrs:mos)	Scaled Score[a]
Baseline	Below 4	1	Below 4	1
BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; NA, not available. [a]The mean (SD) scaled score for healthy age- and sex-matched peers is 15 (5).				

His baseline Z-scores for height (-3.5) and weight (-1.0) were well below average. With only baseline data available, his BOT-2 scaled score, 6MWT (280 meters; NA predicted), and HHD (9%-37% predicted) demonstrated significant delays.

He did not experience any TEAEs that resulted in a change to dosing regimen. No funduscopic examination data were reported. He had an ISR of mild erythema, beginning Day 5 up to the last visit (Month 1), which was ongoing and non-serious. He was negative for anti-asfotase alfa antibodies at baseline.

No further data are available following withdrawal from the study at Month 1.

eResults

eTable 1. Change in RSS scores with asfotase alfa treatment

	Baseline	Month 6	Year 1	Year 2	Year 3	Year 4	Year 5
Variable	**Historical Control Patients**						
N	16	16	16	15			
RSS, mean (SD)	1.44 (0.96)	1.31 (1.05)	1.19 (1.03)	1.43 (1.19)			
RSS, median (min, max)	1.00 (0.0. 3.5)	1.25 (0.0. 4.0)	1.00 (0.0, 4.0)	1.00 (0.5, 5.0)			
RSS Change from baseline, median (min, max)		0.00 (-1.0, 1.5)	-0.50 (-1.0, 1.5)	0.00 (-1.5, 1.5)			
P[A]		0.41	0.16	0.83			
	Asfotase Alfa-treated Patients						
N	12	12	12	12	8	10	10
RSS, mean (SD)	2.75 (1.39)	1.04 (1.23)	1.25 (1.25)	1.04 (1.12)	0.56 (0.68)	0.40 (0.52)	0.15 (0.34)
RSS, median (min, max)	2.75 (0.5, 6.0)	0.75 (0.0, 4.5)	1.00 (0.0, 4.5)	0.50 (0.0, 4.0)	0.50 (0.0, 2.0)	0.25 (0.0, 1.5)	0.00 (0.0, 1.0)
RSS Change from baseline, median (min, max)		-1.50 (-3.5, -0.5)	-1.25 (-3.0, 0.0)	-2.00 (-3.5, 0.5)	-2.50 (-4.0, 1.0)	-2.50 (-4.5, 0.0)	-2.75 (-5.0, -0.5)
P[A]		0.0005	0.001	0.002	NA	NA	NA
P[B]		0.0008	0.007	0.0025			

[A] P value based on within group Wilcoxon signed-rank test for change from baseline.

[B] P value based on Wiilcoxon rank sum test comparing change from baseline of the treatment group with the historical control group.

NA, not available.

eTable 2. TNSALP gene mutation analysis - by patient

Patient Number	Gene Mutation Class	AA Change 1 AA Change 2	Nuc Change 1 Nuc Change 2
1	Compound heterozygote	ALA176THR ASP294ALA	C.526G>A C.881A>C
2	Compound heterozygote	ALA176THR ASP294ALA	C.526G>A C.881A>C
3	Heterozygous	ASP378VAL	C.1133A>T
4	Compound heterozygote	GLU191LYS ASP337GLY	C.571G>A C.1010A>G
5	Compound heterozygote	GLU191LYS ASN417SER	C.571G>A C.1250A>G
6	Compound heterozygote	GLU191LYS ASP294ALA	C.571G>A C.881A>C
7	Compound heterozygote	ALA176THR ALA179THR	C.526G>A C.535G>A
8	Compound heterozygote	GLU84ASP VAL459MET	C.252G>C C.1375G>A
9	Compound heterozygote	GLU191LYS GLY334ASP	C.571G>A C.1001G>A
10	Compound heterozygote	GLU191LYS ALA116THR	C.571 G>A C.346G>A
11[A]	NA	NA	NA
12	Compound heterozygote	GLU191LYS P.327DELPHE	C.571G>A C.978 980DELCTT
13	Compound heterozygote	ALA114THR GLY455SER	C.340G>A C.1363G>A

AA,amino acid; NA, not available; Nuc, nucleotide.

[A] No mutation was detected; under further analysis.

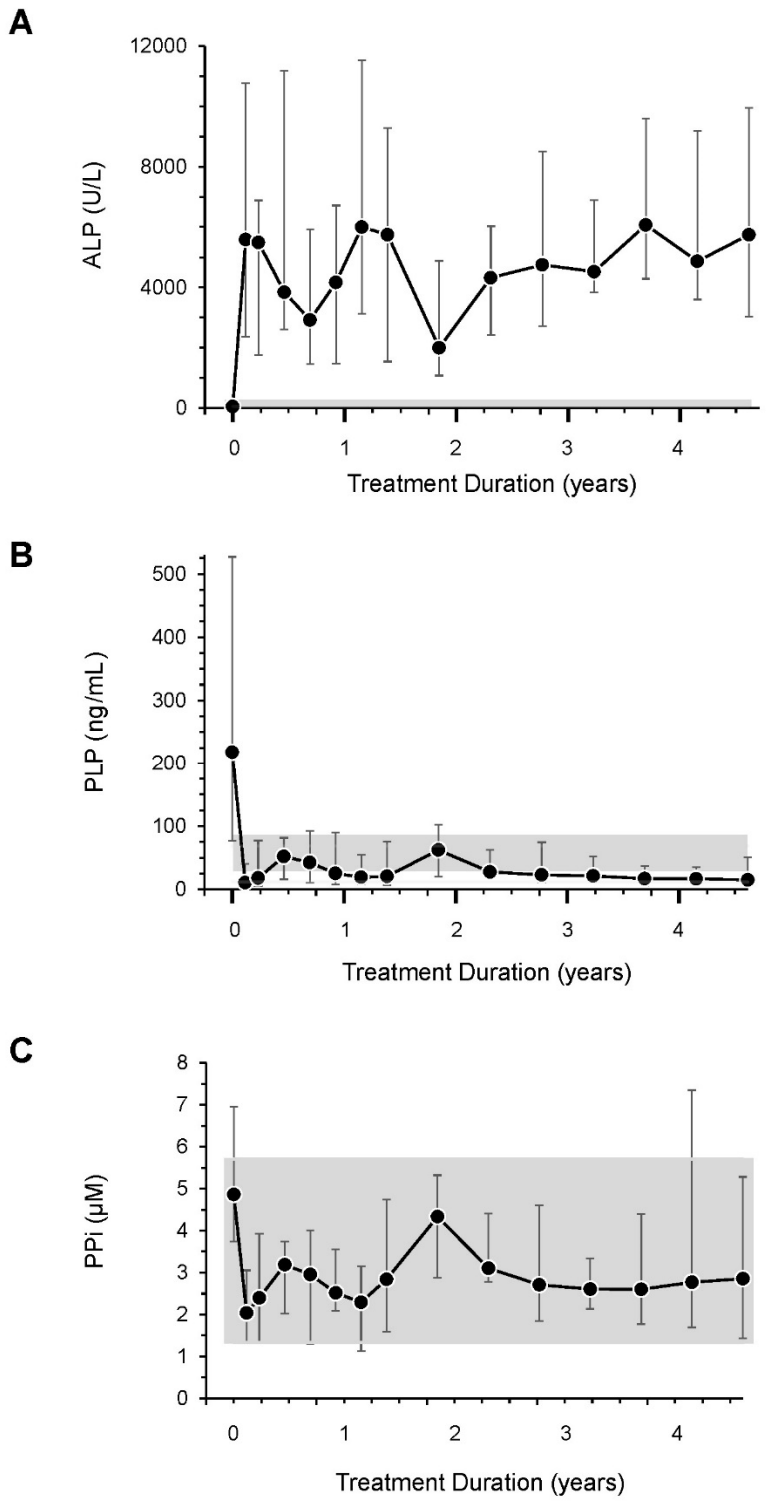
eTable 3. RSS 10-point radiographic scoring method for rickets (5)

Wrist: score both radius and ulna separately		
	Grade	Radiographic features
	0	Normal growth plate without changes of rickets
	0.5	Lucency of metaphyseal margin without fraying or irregularity
	1	Widened growth plate, irregularity of metaphyseal margin but without concave cupping
	1.5	Partial metaphyseal concavity or incomplete fraying of metaphyseal margin
	2	Metaphyseal concavity with fraying of margins
2 bones × 2 points = 4 points possible		

Knee: score both femur and tibia separately		
Multiply the grade in A by the multiplier in B for each bone, then add femur and tibia scores together		
A	Grade	Degree of lucency and widening of zone of provisional calcification
	0	Normal growth plate without changes of rickets
	1	Partial lucency, smooth margin of metaphysis visible
	2	Partial lucency, smooth margin of metaphysis NOT visible
	3	Complete lucency, epiphysis appears widely separated from distal met
B	Multiplier	Portion of growth plate affected
	0.5	≤1 condyle or plateau
	1	2 condyles or plateaus
2 bones × 1 point × 3 points = 6 points possible		

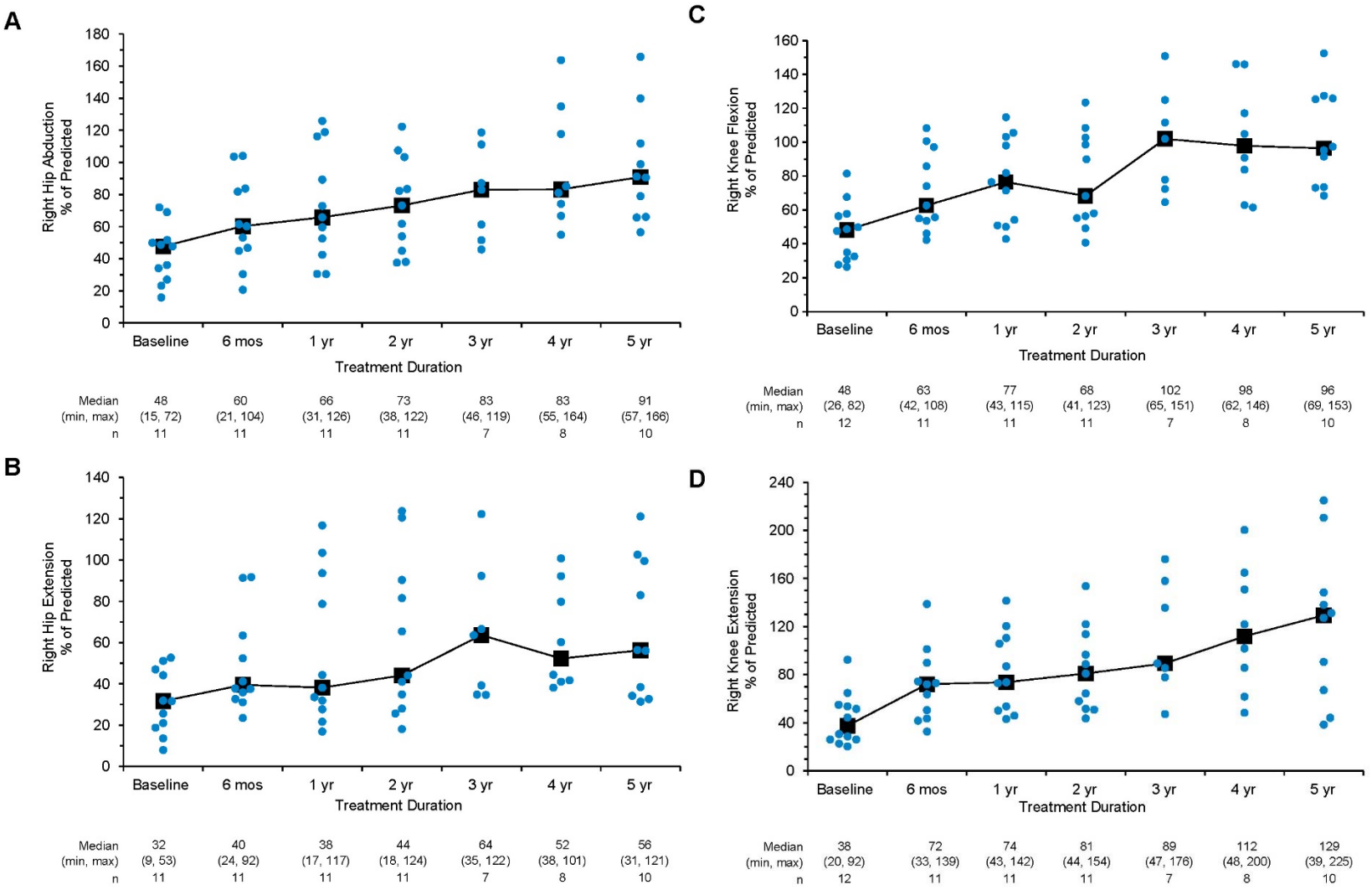
Total: 10 points possible that would indicate very severe rickets

eFigure 1. Serum ALP, PLP, and PPi levels



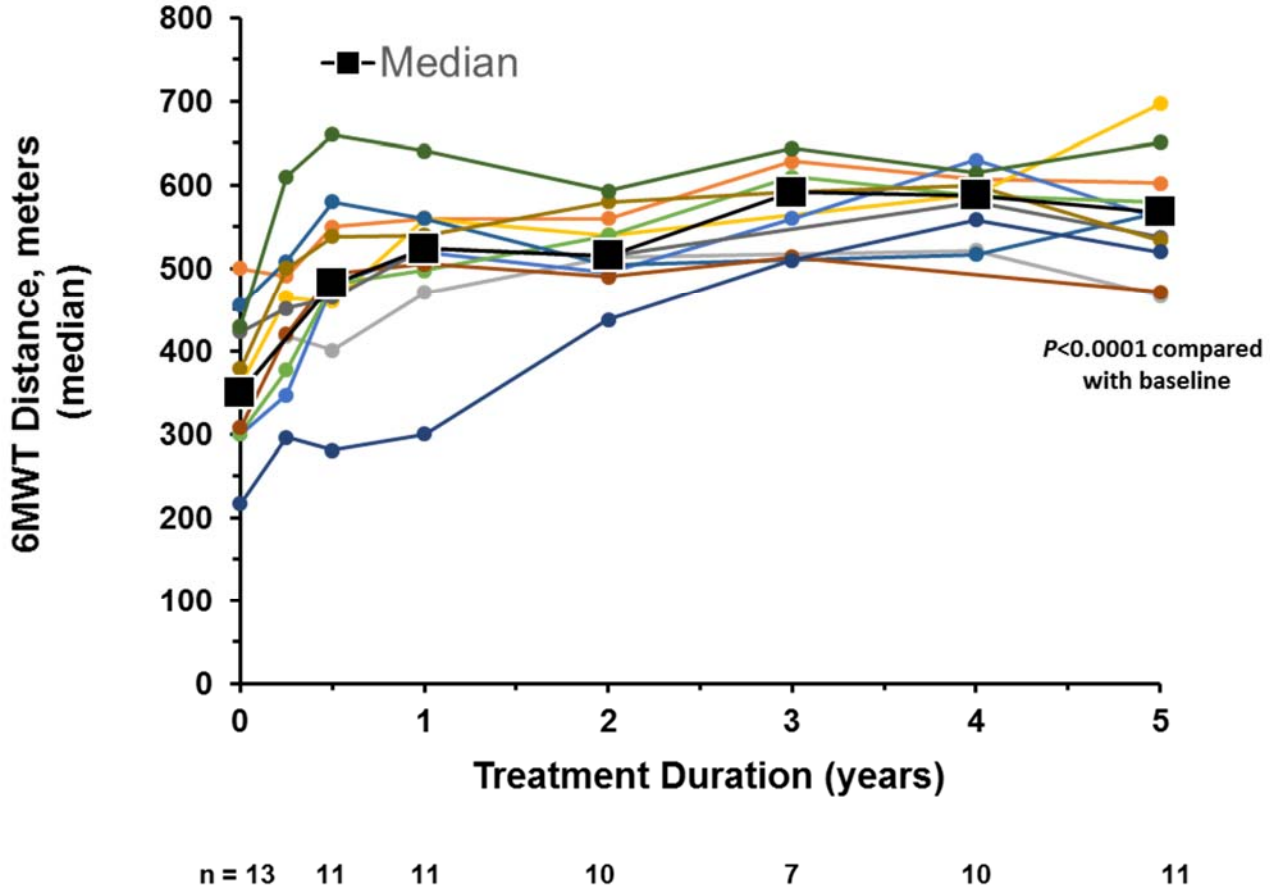
Values are median and the error bars represent the minimum and maximum. Gray zones are normal ranges.

eFigure 2. Effect of asfotase alfa on (A and B) hip and (C and D) knee strength



The minimum and maximum at each timepoint are presented beneath each plot.

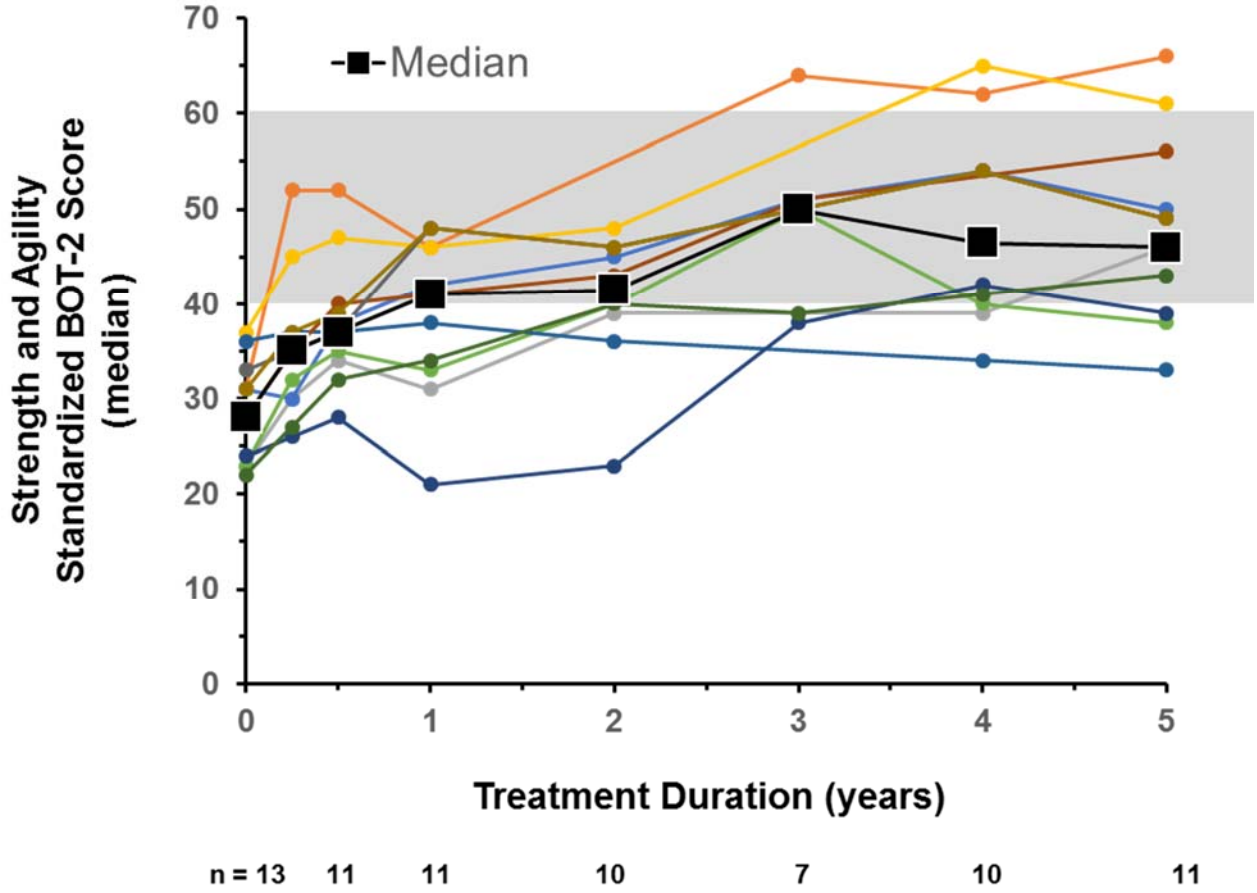
eFigure 3. 6MWT - individual patient and overall data



6MWT, six minute walk test.

P-value for t-test assessing whether the mean change from baseline differed from 0.

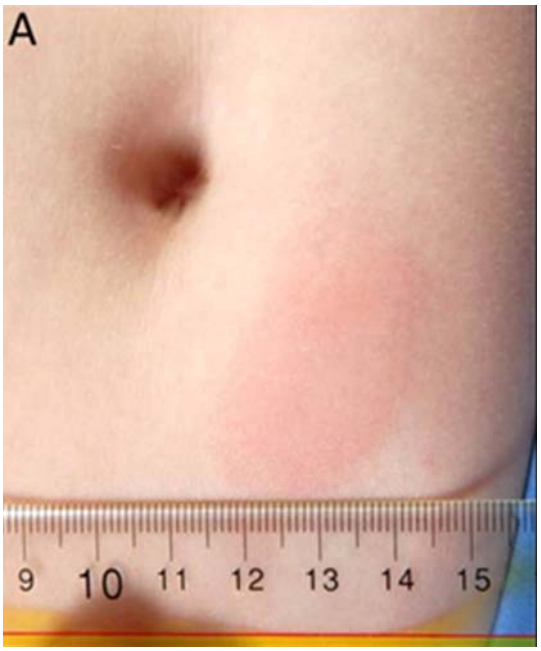
eFigure 4. BOT-2 - individual patient and overall data

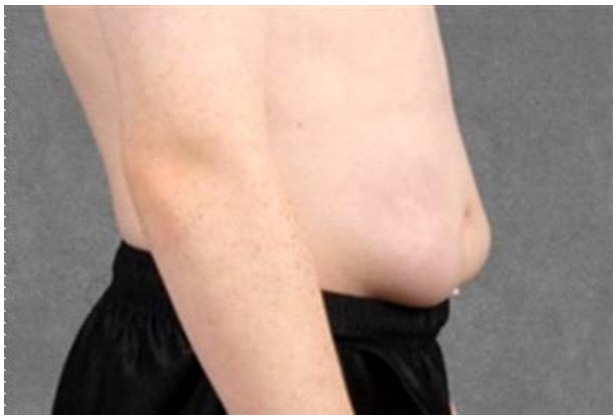


BOT-2, Bruininks-Oseretsky Test of Motor Proficiency, Second Edition.

BOT-2 Strength and Agility composite standard score (data in bars represent median). Gray shading: mean (SD) Strength and Agility score for healthy peers is 50 (10).[5] Incomplete data for Patient 1 is indicated by a broken line from 1-3 years.

eFigure 5. Representative examples of injection-associated reactions (A) erythema (abdomen) and (B) lipohypertrophy (abdomen)





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5. Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. Radiographic scoring method for the assessment of the severity of nutritional rickets. *J Trop Pediatr.* 2000;46(3):132-139.