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Hypophosphatasia: Seven-Year Outcomes for Life-Threatening Disease in Infants and Young Children Treated With Asfotase Alfa

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Research in context

Evidence before this study

Hypophosphatasia is the inborn-error-of-metabolism characterized by low activity of the alkaline phosphatase isoenzyme found abundantly in bone and liver. During growth, this deficiency can lead to rickets. Asfotase alfa, an enzyme replacement therapy for hypophosphatasia, was approved multinationally in 2015. Prior management of this disease involved supportive care. In 2012, we reported 1-year findings from an open-label, multinational study that evaluated the safety and efficacy of asfotase alfa for infants and young children with the life-threatening perinatal or infantile forms of this heritable metabolic bone disease (Whyte et al. *N Engl J Med.* 2012;366(10):904-13). Significant healing of the skeleton was accompanied by improved respiratory function and developmental milestones, and this biologic was generally well tolerated. In 2016, these same patients had improved survival and respiratory outcomes compared with historical controls (Whyte et al. *J Clin Endocrinol Metab.* 2016;101(1):334-42). In another 2016 study, older children with symptomatic hypophosphatasia demonstrated sustained improvement in skeletal mineralization with most achieving normal values for age- and sex-matched peers in growth, strength, and motor function during 5 years of asfotase alfa treatment (Whyte et al. *JCI Insight.* 2016;1(9):e85971).

Added value of this study

Here, the long-term impact of asfotase alfa treatment is presented for those infants and young children with life-threatening hypophosphatasia given a median of 6.6 years of

therapy. The early improvements were sustained for up to 7 years of treatment. Typically, the better skeletal mineralization during the first 6 months of treatment was followed by withdrawal of respiratory support, and then associated with improved motor and cognitive function persisting until study end. Although most patients had required prolonged pulmonary support, all nine who completed the study no longer needed it after Year 4. For most patients, improvements in length/height and weight Z-scores indicated catch-up growth. Substantially better gross motor, fine motor, and cognitive function could match healthy peers. Asfotase alfa was generally well tolerated, with the most common treatment-emergent adverse events consistent with sequelae of hypophosphatasia. No evidence of resistance to the therapy emerged.

Implications of all the available evidence

This now completed study documents long-term safety and efficacy of asfotase alfa treatment for infants and young children with life-threatening hypophosphatasia. The findings complement observations from the 5-year study of treatment of older children with hypophosphatasia. For life-threatening pediatric-onset hypophosphatasia, prompt diagnosis and commencement of asfotase alfa treatment can rescue such patients and give them enjoyable health.

ABSTRACT

Background: Our Phase 2, open-label study of 11 infants and young children with life-threatening perinatal or infantile hypophosphatasia (HPP) demonstrated 1-year safety and efficacy of asfotase alfa, an enzyme replacement therapy. We report outcomes over ~7 years.

Methods: Patients received asfotase alfa (1 mg/kg thrice weekly subcutaneously; adjusted to 3 mg/kg thrice weekly if required). HPP skeletal manifestations were evaluated on the Radiographic Global Impression of Change (RGI-C) scale (−3=severe worsening; +3=complete/near complete healing). Respiratory support, growth, and cognitive and motor function were also evaluated.

Findings: Ten patients completed a 6-month treatment period and entered an extension; nine received asfotase alfa for ≥6 years and completed the study, with four treated >7 years. Skeletal healing was sustained over 7 years of treatment; all evaluable patients had RGI-C scores ≥+2 at Years 6 and 7. No patient who completed the study required respiratory support after Year 4. Weight Z-scores improved to within normal range from Year 3 to study end; length/height Z-scores improved but remained below normal. Age-equivalent scores on Gross Motor, Fine Motor, and Cognitive subscales of the Bayley Scales of Infant and Toddler Development also improved. Treatment was generally well tolerated; adverse events were similar to those previously published.

Interpretation: Patients with perinatal or infantile HPP treated with asfotase alfa for up to 7 years showed early, sustained improvements in skeletal mineralization. Respiratory function, growth, and cognitive and motor function also improved. Asfotase alfa is safe and effective in perinatal/infantile HPP.

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Keywords: alkaline phosphatase; ambulation; enzyme replacement; inorganic pyrophosphate; metabolic bone disease; osteomalacia; pyridoxal 5'-phosphate; rickets

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INTRODUCTION

Hypophosphatasia (HPP) is the rare, inherited, metabolic bone disease caused by loss-of-function mutation(s) of the *ALPL* gene that encodes the tissue-nonspecific isoenzyme of alkaline phosphatase (TNSALP).[1,2] Low TNSALP activity on cell surfaces results in extracellular accumulation of its substrates, including inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP).[1,3-5] PPi potently inhibits mineralization by blocking hydroxyapatite crystal formation.[6,7] Thus, the superabundance of PPi in HPP often leads to rickets during growth.[6,7] TNSALP dephosphorylates PLP (the principal circulating form of vitamin B6) to pyridoxal, which allows it to cross cell plasma membranes and be rephosphorylated intracellularly to PLP. Thus, vitamin B6–dependent seizures occur in some severely affected babies.[4,7-9] Life-threatening complications in the severe perinatal and infantile forms of HPP can include respiratory failure from rachitic chest deformity and rib fractures, elevated intracranial pressure due to craniosynostosis, and hypercalcemia leading to nephrocalcinosis and renal compromise.[7,10-12] Other potential complications in pediatric HPP include long bone deformity and muscle weakness.[7,10] Perinatal HPP historically has been considered lethal, and infantile HPP has ~50% mortality during the first year of life.[13-15]

Asfotase alfa (Strensiq®; Alexion Pharmaceuticals, Inc., Boston, MA) is a human, recombinant, TNSALP replacement therapy approved multinationally in 2015, typically for pediatric-onset HPP.[16,17] The safety and efficacy of asfotase alfa were first evaluated during our Phase 2, open-label study in pediatric patients ≤3 years of age with life-

threatening perinatal or infantile HPP.[18] This study enrolled 11 HPP patients (5 with perinatal HPP and 6 with infantile HPP) ranging in age from 2 weeks to 3 years for the 6-month initial trial.[18] Patients manifested complications of HPP before 6 months of age, including skeletal abnormalities such as shortened or bowed limbs, rachitic chest deformity, fractures, osteopenia, craniosynostosis, and/or other rachitic findings. All but one patient had failure to thrive, most (82%) required respiratory support, and all had gross motor delay. A single 2 mg/kg intravenous (IV) infusion of asfotase alfa preceded subcutaneous (SC) injections starting at 1 mg/kg thrice weekly. Results from this study published in 2012 showed outcomes after ≥12 months (range: 12–26 mo) of treatment with asfotase alfa.[18] One patient had consent withdrawn on Day 1 because of infusion-associated reactions (IARs), and one patient died of pneumonia and sepsis after 7·5 months of treatment.[18,19] The study met its primary efficacy measure of change in HPP skeletal disease severity from Baseline to Month 6 based on assessment of skeletal radiographs using the validated 7-point (−3=severe worsening; +3=complete/near complete healing) Radiographic Global Impression of Change (RGI-C) scale (median [min, max]: +2·0 [0, +2·3]; p=0·004). Skeletal healing was accompanied by improvements in secondary outcome measures of respiratory and motor function over 1 year of treatment. Asfotase alfa was generally well tolerated.[18]

Herein we report the long-term efficacy (skeletal manifestations, respiratory support, growth, and motor and cognitive function), pharmacodynamics, and safety after approximately 7 years of treatment with asfotase alfa.

METHODS

Study design

Each study site undertook approved research governance and ethics processes to authorize the investigation. Parents and legal guardians signed the consent form before study participation.

The study design, including patient inclusion and exclusion criteria, has been published.[18] The patient numbering scheme used in that publication is continued in our current report. Briefly, this Phase 2, open-label study was conducted at 10 sites (six in the United States, two in the United Kingdom, one in Canada, and one in the United Arab Emirates). In the 6-month primary treatment period, safety and efficacy of asfotase alfa were evaluated in infants and young children (≤3 years of age) with life-threatening perinatal or infantile HPP (clinicaltrials.gov identifier: NCT00744042; EudraCT number: 2008-007406-11). A single 2 mg/kg IV infusion of asfotase alfa preceded 1 mg/kg SC injections of asfotase alfa thrice weekly (total dosage: 3 mg/kg/wk). The dosage could be increased to up to 3 mg/kg thrice weekly (total dosage: 9 mg/kg/wk) after 1 month for lack of efficacy, defined as worsening of failure to thrive, deteriorating pulmonary function, or no radiographic evidence of skeletal improvement. The extension phase (NCT01205152; 2009-009369-32) continued the SC asfotase alfa dosage from the primary treatment period; dosage adjustments were made at each visit for changes in the patient's weight. Additional dosage adjustments (no limits on maximum dosage) were permitted for lack of efficacy or for safety-related concerns. Patients continued to

receive asfotase alfa for up to 7 years total (primary treatment period plus extension phase) or until the product became commercially available, whichever occurred first.

Efficacy outcome measures

Change in HPP skeletal disease: HPP skeletal manifestations were evaluated using sequential radiographs of the chest, wrists, and knees obtained at Baseline; Months 1, 3, 6, and 9; and Years 1, 1·5, 2, 2·5, 3, 4, 5, 6, and 7. At each time point, the same 3 paediatric radiologists independently rated changes compared with Baseline using the RGI-C scale (−3=severe worsening, 0=no change, and +3=complete/near complete healing), which has been validated in pediatric patients with HPP.[20] The mean of the raters' RGI-C scores was calculated for each patient at each time point. A separate rater independently evaluated the radiographs of the wrists and knees at each time point using the 10-point Rickets Severity Scale (RSS; 0=absence of metaphyseal cupping and fraying to 10=severe rickets; maximum of 4 points for wrists and 6 points for knees), developed and validated to assess nutritional rickets in children (mean age: 4·5 years).[21] All raters were blinded to all treatment time points (except Baseline radiographs for RGI-C) and all other patient information.

Respiratory support: Use of supplemental oxygen, continuous positive airway pressure (CPAP), biphasic positive airway pressure (BiPAP), and mechanical ventilation was documented at each study visit.

Growth: Length/height, weight, and head circumference were recorded at study visits. Z-scores for length/height and weight were determined using Centers for Disease Control and Prevention growth charts for age- and sex-matched healthy infants and children.[22] Head circumference Z-scores were calculated using World Health Organization (WHO) formulae.[23]

Motor and cognitive function: Depending on the patient's age and functional abilities at individual visits, each site's physical therapist, in consultation with the Medical Monitor, determined the appropriate assessment or combination of assessments of motor and cognitive development, which included the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III); the Peabody Developmental Motor Scales, Second Edition (PDMS-2); or the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) (**Figure 1**). Patients ≤42 months of age, or older but with severe developmental delays, were assessed using the Gross Motor, Fine Motor, and Cognitive subscales of the BSID-III.[24] If patients demonstrated cognitive age-equivalence of 42 months, such testing was discontinued. Patients 43–71 months of age considered to have evaluable functional abilities were studied using the Locomotion subtest of the PDMS-2, an assessment of gross motor skills.[25] Patients ≥72 months of age with evaluable functional abilities completed the Running Speed and Agility and Strength subtests of the BOT-2, an assessment of gross motor proficiency.[26] Licensed physical therapists, or their local equivalents, conducted the assessments at Baseline (BSID-III only), Months 3 (BSID-III only) and 6, Year 1, and every 6 months thereafter.

When possible, these functional assessments occurred before same-day invasive tests or examinations that could tire the patient.

Pharmacodynamic outcome measures

Blood was collected for assay of serum alkaline phosphatase (ALP) activity, plasma PPi and PLP concentrations, and serum intact parathyroid hormone (PTH) at Baseline; Months 3, 6, and 9; Year 1; and every 6 months thereafter. Treatment samples were collected before asfotase alfa dosing and after patients had fasted ≥4 hours. The tubes for blood sampling of PPi and PLP contained levamisole to inhibit the high ALP activity from asfotase alfa. Laboratory samples were managed by a central facility (Covance, Inc., Indianapolis, IN, USA, and Geneva, Switzerland). PPi analyses were conducted by Alexion Montreal Corporation (Montreal, Quebec, Canada) and Charles River Laboratories (Senneville, Quebec, Canada). At first, PLP analyses were conducted at local laboratories but were later performed at 2 central laboratories (ARUP Laboratories, Inc., Salt Lake City, UT, USA, and Biotrial Bioanalytical Services, Inc., Laval, Quebec, Canada). Reported PLP results were not censored for vitamin B6 supplementation, which can markedly increase PLP in patients with HPP.[27] Additional details regarding the PPi and PLP assays are provided in the **Supplementary Appendix.**

Safety and tolerability

Adverse events (AEs), including injection site reactions (ISRs) and injection-associated reactions (IARs), were continuously monitored. ISRs were defined as AEs localized to the site of asfotase alfa administration, and IARs were defined as systemic signs, symptoms, or findings (e.g., chills, cough, erythema) occurring within 3 hours after asfotase alfa administration. The site investigator assessed the “possible,” “probable,” or “definite” relationship of AEs to the study drug. Additional safety assessments included physical examination findings, laboratory values (including calcium and phosphate), and anti–asfotase alfa antibody testing results (the latter performed by Cambridge Biomedical Inc., Boston, MA, USA, and PPD Laboratories, Richmond, VA, USA). Patients were assessed for ectopic calcification by periodic funduscopic examinations (changed to full ophthalmologic examinations by protocol amendment after ~1·5 years) and periodic renal ultrasounds.

Statistical analyses

All efficacy and safety analyses were performed using the full analysis (FA) population, which included all patients who received any asfotase alfa. Some analyses were repeated using the per protocol (PP) population, which included all patients who received any asfotase alfa and did not have any major protocol deviations considered to potentially influence treatment effect. Because of the timing of study visits, annual time points were approximated, with 48 weeks defined as 1 year.

Median (min, max) values were calculated for the RGI-C and RSS scores; length/height, weight, and head circumference raw values and Z-scores; and BSID-III scaled scores over time. A 1-sample Wilcoxon signed-rank test using a 2-sided alpha of 0·05 was used to test whether the median RGI-C score at each time point differed from 0 (i.e., no change). The proportion of patients with RGI-C scores of +2 or +3 (RGI-C "responders") was calculated for each time point. Mean changes from Baseline in Z-scores for length/height and weight were analyzed using a 1-sample *t*-test. Age-equivalent and scaled (or standard) scores were calculated for each subscale/subtest of the BSID-III, PDMS-2, and BOT-2. Scaled or standard scores for each were then compared with the normative mean (SD) values for healthy age-matched peers, which were 10 (3) for the BSID-III scaled score,[24] 10 (3) for the PDMS-2 standard score,[25] and 15 (5) for the BOT-2 Running Speed and Agility subtest scaled score.[26] These functional measures were exploratory and therefore not analyzed statistically.

Pharmacodynamic and safety outcome measures are summarized descriptively.

Role of the funding source

Alexion Pharmaceuticals, Inc., was the funding source and was involved in all stages of the study conduct and analysis.

RESULTS

Patients

Eleven patients were enrolled and received at least one dose of asfotase alfa (median age at enrollment: 30 weeks [min: 3; max: 158 [~3 y]; sex: 64% female). All ten patients who completed the 6-month primary treatment period entered the extension phase; nine received asfotase alfa for ≥6 years and completed the study, with four of the nine treated for >7 years. Patients were enrolled and received treatment between October 2008 and August 2016. The median duration of treatment for the 11 enrolled patients was 6·6 years (range: 0·003–7·5). Patient demographics, Baseline characteristics, and efficacy and safety outcomes for nine patients through ≥1 year of treatment and detailed case reports of all 11 enrolled patients were published.[18] Updated narratives for all patients who completed the study are provided in the on-line **Supplementary Appendix** to this article.

Four patients had major protocol deviations considered to potentially influence treatment effect and were therefore excluded from the PP population. Two did not meet the ALP eligibility criterion (ALP activity ≤3 SD below the mean for age). One did not meet the failure to thrive eligibility criterion (too young developmentally for assessment) and did not receive a weight-adjusted dose of asfotase alfa (received 8 mg SC injections thrice weekly) from approximately Week 9 until his death at Week 23, with his last recorded weight being 5·7 kg. One patient had dosage increases for failure to thrive on Day 22 (from 1 mg/kg thrice weekly to 1·5 mg/kg [one dose only]) and on Day 24 (to

3 mg/kg thrice weekly); dosage increases were not permitted per protocol before completion of 1 month of treatment.

Because primary efficacy results (RGI-C scores at Month 6) were similar between the FA and PP populations (data not shown), we present results after 6–7 years of asfotase alfa treatment for the FA population only.

Efficacy Outcome Measures

Changes in skeletal manifestations of HPP

Median RGI-C scores documented improvements in HPP skeletal disease as early as Month 3 that were typically sustained over 7 years of treatment, with statistically significant ($p<0{\cdot}05$) gains at most visits (**Figure 2A**). The proportion of evaluable patients with RGI-C scores ≥+2 (responders) was 89% (8/9) at Year 1 and 100% (7/7) at Year 7. The highest possible RGI-C score of +3 was achieved by four patients, with three first achieving a +3 by Year 1 and one first achieving a +3 at Year 2; all four maintained scores ≥+2 at all time points thereafter.

Median RSS scores confirmed that the improvements documented as early as the sixth month[18] were sustained over 7 years of treatment, with significant ($p<0{\cdot}05$) decreases indicating improvement from Baseline at most visits (**Figure 2B**).

Most patients had an overall RGI-C score at Last Assessment ranging from +2 (substantial healing) to +3 (complete/near complete healing). The substantial

improvements in radiographic findings for the wrists and knees in 2 patients treated with asfotase alfa over 7 years of treatment are illustrated in the representative images in **Figure 3**. Illustrative radiographs for all patients are included in the **Supplementary Appendix**.

Respiratory support

Duration of respiratory support was published in 2016 for the six patients from this study requiring CPAP, BiPAP, or mechanical ventilation, with a maximum follow-up of 6 years.[15] **Figure 4** summarizes respiratory support over time for all 11 patients. At Baseline, 45% (5/11) required respiratory support, with 27% (3/11) mechanically ventilated, 9% (1/11) receiving CPAP, and 9% (1/11) receiving supplemental oxygen. By Year 2, 33% (3/9) required respiratory support, with 11% (1/9) mechanically ventilated and 22% (2/9) receiving just supplemental oxygen. From 4·5 years of treatment through study end, none of the nine patients required respiratory support (including supplemental oxygen).

Growth

Median (min, max) length/height was 56·5 (39·0, 83·0) cm at Baseline (n=11) and 112·5 (88·1, 123·0) cm at Year 7 (n=7). The median length/height Z-score was higher than at Baseline from Month 6 through Year 7, although the value remained >2 SD below the mean for healthy age- and sex-matched peers at all time points (**Figure 5**). Overall, four of nine patients had Z-scores within the normal range at Last Assessment. The mean

increase from Baseline in length/height Z-score was statistically significant at Year 3 (p=0·0385) and Year 4·5 (p=0·0346), but not at other time points.

Median (min, max) weight was 4·1 (2·1, 9·2) kg at Baseline (n=11) and 19·8 (15·1, 31·4) kg at Year 7 (n=7). Median weight Z-scores increased to within 2 SD of the mean for healthy age- and sex-matched peers at most time points from Years 3 through 7 (**Figure 5**). The mean increase from Baseline in weight Z-score was statistically significant at Year 3 (p=0·0096) and Year 4·5 (p=0·0074), but not at other time points.

Median (min, max) head circumference was 41·5 (33·0, 47·6) cm at Baseline (n=11) and 50·5 (44·5, 51·3) cm at Year 7 (n=7). Head circumference Z-scores remained stable, with a median (min, max) value of −1·01 (−4·0, 0·8) at Baseline (n=11) and −1·34 (−4·1, 1·0) at Last Assessment (n=10; WHO criteria allow for calculation of head circumference Z-scores for patients aged ≤5 years).

Motor and cognitive function

BSID-III: At Baseline or first assessment, 82% (9/11) of patients had BSID-III Gross Motor scaled scores of 1 (3 SDs below the normative mean). Nine patients had serial BSID-III assessments (two patients had one assessment each because of discontinuation and death) (**Table S1**). All nine showed improvements in age-equivalent scores on the Gross Motor, Fine Motor, and Cognitive subscales (**Table S2**). Median (min, max) scaled Gross Motor scores improved from 1·0 (1, 8) at Baseline to 6·0 (2, 8) at Year 3 (normative mean [SD]: 10 [3]; **Figure 6A**), indicating motor skill improvement

and less developmental delay. Median scores on the Fine Motor and Cognitive subscales were low at Baseline but normalized at Years 2 and 3 (**Figure 6A**).

PDMS-2: Eight patients advanced to complete serial PDMS-2 assessments (**Table S1**). All demonstrated continued motor skill acquisition on the PDMS-2 Locomotion subtest (i.e., increased age-equivalent scores [**Figure S1**]). Among them, seven had standard scores >1 SD below the normal reference range (score: <7) when they first completed the assessment; five achieved scores within 1 SD of normal (**Figure 6B**).

BOT-2: Eight patients transitioned to the BOT-2 and completed at least one BOT-2 assessment (**Table S1**); all had received asfotase alfa for ≥5 years when first tested. Seven had initial scaled BOT-2 Running Speed and Agility subtest scores >1 SD below normal (scaled score: <10) (**Figure 6C**). Three achieved normal scaled scores (≥10) by study end. All six who completed serial BOT-2 assessments had increased age-equivalent BOT-2 scores during treatment (**Table S3**).

Pharmacodynamic Outcome Measures

Baseline serum ALP activity was low (median [min, max]: 21 [9, 46] U/L) in the nine evaluable patients, increased by approximately 100-fold by Day 2 after the single IV infusion of asfotase alfa (2990 [449, 8007] U/L; n=9), and remained elevated thereafter with SC dosing (e.g., 5304 [1812, 10085] U/L at Year 1 [n=8][18] and 4143 [2267, 6792] U/L at Year 7 [n=7]).

Baseline plasma concentrations of PPi were elevated in four of eight evaluable patients (median [min, max]: 5·2 [2·9, 10·5] μM; normal range for patients aged 0–12 years: 1·33–5·71 μM) (**Figure S2A**). Median PPi levels decreased for all nine patients tested at Month 3 of treatment (2·6 [1·0, 4·4] μM), and remained decreased relative to Baseline at other study visits including Year 7 (4·6 [2·1, 10·2] μM; n=7), with the exception of isolated fluctuations for individual patients.

Baseline plasma concentrations of PLP were elevated in all nine evaluable patients (median [min, max]: 421·0 [100·0, 880·0] ng/mL; normal range for patients aged 0–5 y: 11·8–68·4 ng/mL) (**Figure S2B**). Median PLP levels stayed within the normal range from Month 6 (47·6 [16·4, 1510·0] ng/mL; n=10) to Year 7 (38·8 [19·1, 161·0] ng/mL; n=7; normal range for patients aged 5–18 y: 5·7–61·2 ng/mL). Only one patient's PLP value failed to correct at any time during the study (range: 81·3–960 ng/mL); this patient was not receiving vitamin B6 supplementation. Overall, data appeared similar when patients receiving vitamin B6 supplementation were excluded from the analysis (not shown).

Serum concentrations of PTH over the course of treatment are shown in the Supplementary Appendix (**Figure S3**).

Safety

As previously published,[18] one patient withdrew because of AEs during the initial IV infusion of asfotase alfa and one patient died from sepsis at ~8 months of age after 7·5

months of therapy. No additional deaths or discontinuations occurred. All 11 patients experienced at least one treatment-emergent AE (TEAE). **Table 1** summarizes the TEAEs occurring in >25% of patients, regardless of relationship to asfotase alfa. The most common were pyrexia (73% [8/11]), upper respiratory tract infection (73% [8/11]), craniosynostosis (64% [7/11]), pneumonia (64% [7/11]), constipation (55% [6/11]), otitis media (55% [6/11]), and vomiting (55% [6/11]). Most events were mild (76% [605/794]) or moderate (19% [151/794]) in severity; eight patients (73%) had severe TEAEs (38 events; **Table S4**). Most events were also considered by investigators to be unrelated to the study drug (84% [664/794]). Those assessed by investigators as possibly, probably, or definitely related to asfotase alfa in more than two patients were injection site erythema (n=4), irritability (n=3), pyrexia (n=3), and vomiting (n=3). Ten (91%) of the 11 patients experienced 79 serious TEAEs (**Table S4**). Those considered by the investigator to be related to asfotase alfa were severe chronic hepatitis (n=1; this event occurred concurrently with use of montelukast and resolved upon discontinuation of montelukast), moderate immediate postinjection reaction (IAR: abdominal pain, skin erythema, dizziness, headache, and chills; n=1), and severe craniosynostosis with severe conductive deafness (n=1).

Four patients had a total 10 AEs considered by the investigators as possibly reflecting hypersensitivity that were therefore designated IARs. Most (8/10) occurred on Day 1 in conjunction with the initial IV infusion. The only IAR reported for more than one patient was pyrexia (n=3). All IARs were mild or moderate in severity; none were life-threatening. The one patient who withdrew from the study experienced IARs of

piloerection, pyrexia, and chills during the IV infusion. Blood complement testing was performed in two patients with IARs; neither had a clinically significant result.

Seven patients (64%) experienced 78 ISRs; the majority (60% [47/78]) occurred in two patients. No severe or serious ISRs were reported. One patient had TEAEs of injection site calcifications observed radiographically in the soft tissue lateral to the left and right hip joints after receiving asfotase alfa injections deeply and repeatedly there for approximately 1 year (dosage at the time of the AE: 2 mg/kg thrice weekly). The calcifications were considered possibly drug-related, treated by rotating the injection sites, and resolved by study end. Three patients had TEAEs of lipohypertrophy, all mild or moderate in severity, occurring after ≥2 years of treatment, and ongoing at study end.

Two patients had TEAEs of seizures during treatment; one had pyridoxine-dependent seizures before and during the study. Six patients had fractures during the study; all but one had a prestudy history of fractures. Seven patients had 13 craniosynostosis-related events that were all moderate or severe and, in all but one patient, considered unrelated to asfotase alfa treatment. Four patients underwent surgery for craniosynostosis.

One patient developed mild TEAEs of ectopic calcifications in the conjunctiva approximately 6·5 years after starting treatment; no action was taken. The findings were considered to be possibly related to asfotase alfa treatment and were ongoing at study end. Two patients had TEAEs of nephrocalcinosis, observed when the asfotase alfa dosage was 2 mg/kg thrice weekly. The first patient had a history of bilateral

nephrocalcinosis at Baseline, and the TEAE was first documented at the first study renal ultrasound at Week 48. No action was taken, and the event was ongoing at study end. The second patient had no history of nephrocalcinosis at Baseline, but a questionable calcium deposit was reported in the first renal ultrasound at Month 6. Nephrocalcinosis was first reported as a TEAE at approximately Year 3. The patient was treated with oral potassium citrate twice daily for 3 months. Subsequent renal ultrasounds indicated small calcium deposits at Years 4·5, 5, and 5·5 that did not meet criteria for nephrocalcinosis and were gone from Year 6 through study end.

Serum concentrations of calcium and phosphate over the course of treatment are shown in the Supplementary Appendix (**Figure S3**).

Eight of 10 evaluable patients (80%) tested positive for anti–asfotase alfa antibodies (maximum titer: 2048) over the course of treatment; 5 tested positive for neutralizing antibodies. No dosage adjustments were made based on the presence of antibodies, and the antibodies had no apparent effect on pharmacodynamic outcomes, improvements in skeletal manifestations, or other outcome measures.

DISCUSSION

Herein we report on the long-term (up to 7 years) safety and efficacy of asfotase alfa treatment for pediatric patients with life-threatening HPP. Although rates of mortality historically have been high in patients with perinatal and infantile HPP, individuals in our study who began therapy as infants or young children showed rapid and substantial

improvements in skeletal mineralization and then respiratory, motor, and cognitive function documented at 1 year of treatment with asfotase alfa.[18] These improvements persisted over 7 years of therapy.

Pharmacodynamic results showed that decreases in the plasma concentrations of TNSALP substrates (i.e., PPi and PLP) achieved by 6 months of treatment[18] persisted throughout the study, except for transient elevations in median PLP concentrations. Moreover, plasma concentrations of PPi with treatment with asfotase alfa were above or near the lower limit of normal and did not decrease to subnormal levels. Low PPi has been associated with increased risk of vascular and other forms of ectopic calcification in animal models and certain patient populations.[28-30]

Radiographic assessments of HPP skeletal disease, made using two validated scales (RGI-C[20] and RSS[21]), confirmed improvement in all evaluable patients as early as Month 6 of treatment.[18] These patients had severe rickets at Baseline, with a median RSS of 8·3. After 4 years of treatment, this was reduced to a median RSS score of 0·5, which represents near absence of metaphyseal cupping or fraying. On the RGI-C scale, which provides a broader assessment of the skeletal features of pediatric HPP, scores of at least +2, indicating substantial healing, were reached during 6 months of treatment and were sustained through Year 7. It may be that with longer treatment, these still prepubertal children will experience further skeletal healing.

Further, the skeletal improvements were associated with sustained improvements in respiratory status. As shown in **Figure 4**, although none of the nine patients required respiratory support from Year 4 through study end, it is important to appreciate that several as babies or young children required prolonged support to achieve this outcome. An expanded cohort of 39 pediatric patients with perinatal/infantile HPP that included those enrolled in the current study (n=11) or in another multicenter, multinational, open-label study (n=28; age ≤5 years) has been assessed for respiratory function and survival during treatment with asfotase alfa (median duration: 2·7 y).[15] These 39 treated patients were compared with 48 untreated historical control patients of similar age and HPP characteristics. Among the 39 treated patients, 21 (54%) required ventilator support: 14 (36%) at Baseline and an additional 7 (18%) soon after initiation of therapy.[15] Among the 48 historical controls, 20 (42%) required some form of respiratory support. Kaplan-Meier estimated survival at 5 years was significantly better for the treated patients (82%) than the historical controls (27%; $p<0·0001$).[15] Among the 21 treated patients ever requiring respiratory support, 16 (76%) survived and 12 (57%) were weaned from respiratory support. Improved skeletal mineralization in treated patients was associated with improved respiratory status, with RGI-C scores ≥+2 (substantial or near complete/complete healing) achieved by all who were weaned from respiratory support.[15] The results of our study are also consistent with those of an open-label, study conducted by Kitaoka et al in 2017, who reported improved survival, skeletal mineralization, and respiratory status in 13 Japanese patients with HPP (median [min, max] age at Baseline: 91 d [0 d, 34 y]) treated with asfotase alfa.[31]

Long-term evaluation of the current cohort of nine treated children provided evidence of catch-up growth in some patients. Improvements were observed as early as Month 6 in median length/height Z-scores and Year 1 in median weight Z-scores. Median weight Z-scores normalized from Year 3 through study end, whereas median length/height Z-scores generally improved but remained below normal throughout the study. Approximately half of the patients had craniosynostosis requiring surgery, and two patients were found to have scoliosis (see **Supplemenaty Appendix**), which may have lowered their length/height Z-scores.

BSID-III assessments indicated profound developmental delays at Baseline. Gross Motor impairment exceeded Fine Motor impairment. Cognitive scores were low at Baseline and increased rapidly. However, as BSID-III Cognitive assessment depends on motor stability in the trunk, head control, visual ability, and ability to manipulate toys, scores may have been artificially low and improved when the children were able to sit independently, emphasizing the importance of increased strength and bone stability for gross motor and global development. All nine treated patients had substantial improvements in age-equivalent scores on the BSID-III Gross Motor (e.g., head control, rolling, sitting, walking), Fine Motor (e.g., manipulating blocks, holding cup, cutting with scissors), and Cognitive (e.g., discriminating and classifying objects) subscales. Most also showed increases in scaled scores, indicating catch up to healthy peers in acquisition of new motor and cognitive skills. Eight patients completed skills assessed by the BSID-III and then advanced to the PDMS-2 Locomotion subtest, which evaluates tasks such as jumping, climbing stairs, running, and skipping. Eight children further

advanced to the BOT-2 Running Speed and Agility subtest, which in itself reflects the development of skills typical of school age children (e.g., shuttle run, hopping). Previously, we reported significant improvements for children aged 6–12 years at baseline (n=13) with severe HPP in growth ($p \leq 0{\cdot}0088$) and motor function ($p \leq 0{\cdot}01$) over 5 years of asfotase alfa treatment during a Phase 2, open-label study and its extension.[32]

Asfotase alfa continued to be generally well tolerated in this study. No deaths or safety-related discontinuations of therapy occurred after the one death and one study withdrawal discussed in our 2012 publication.[18] The most common TEAEs generally reflected typical signs, symptoms, or complications of HPP, as well as infections that commonly occur in healthy young children. Ophthalmologic examinations, renal ultrasound, and anti–asfotase alfa antibody testing revealed no additional significant issues associated with this treatment. Seven patients had 13 craniosynostosis-related events. This was not unexpected, as craniosynostosis is a common complication of HPP[11] and would not be expected to reverse with asfotase alfa treatment. In a natural history study of patients with severe perinatal and infantile HPP, the reported incidence of craniosynostosis was 61%.[33]

Monitoring guidance for patients with HPP receiving treatment with asfotase alfa was published in 2017 by Kishnani et al.[34] In brief, recommendations for safety monitoring include ISRs and any hypersensitivity reactions and lipodystrophy, as well as events of special interest that in severely affected patients can include

hypercalcemia/hypocalcemia, craniosynostosis, ectopic calcifications of the conjunctiva, and nephrocalcinosis. Injection site lipohypertrophy and atrophy can be prevented or minimized by rotating injection sites among the abdominal, deltoid, and thigh areas.[34] Though patients severely affected by HPP may have hypercalcemia,[18] improvements in skeletal mineralization can require an increase in calcium intake upon initiation of asfotase alfa treatment (i.e., hungry bone syndrome). Therefore, it is important in such patients to monitor serum calcium and PTH and to provide additional calcium as needed.

Our study had several limitations. Understandably, it was uncontrolled as life-threatening HPP was present, and it involved a small number of patients manifesting the most severe forms of this rare inborn error of metabolism. However, the improved survival documented here for perinatal/infantile HPP treated with asfotase alfa was consistent with that found in a subsequent investigation of a larger cohort of similarly treated patients that included a matched historical control group.[15] Furthermore, motor and cognitive function were assessed in this current study using different instruments, sometimes sequentially, based on the patient's age, functional capability, and physical status. Lastly, age-equivalent or standard scores may not always capture functional improvements observed through increases in raw point scores.

CONCLUSION

Infants and young children with life-threatening perinatal/infantile HPP treated with asfotase alfa before or at age 3 years showed substantial early improvements in

skeletal mineralization and respiratory function, followed by improved weight and motor and cognitive function, all sustained up to 7 years of treatment. Asfotase alfa was generally well tolerated.

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Declaration of interests

Michael P Whyte was the principal clinical study investigator and received honoraria, travel support, and research grant support from Alexion Pharmaceuticals, Inc.

Jill H Simmons was a clinical study investigator and received honoraria and travel support from Alexion Pharmaceuticals, Inc.

Scott Moseley and **Kenji Fujita** are employees of and may own stock/options in Alexion Pharmaceuticals, Inc., which sponsored the study.

Nicholas Bishop was a clinical study investigator and received grant and/or research support from Alexion Pharmaceuticals, Inc.

Nada J Salman was a clinical study investigator, the study was sponsored by Alexion Pharmaceuticals, Inc., through a hospital agreement.

John Taylor was a clinical study investigator and an employee of Prevea Health Clinic, which at the time of the study, was owned by HSHS St. Vincent Hospital, which received grant and/or research support from Alexion Pharmaceuticals, Inc.

Dawn Phillips was a consultant for Alexion Pharmaceuticals, Inc., at the time of the study and had received funding and travel support from Alexion Pharmaceuticals, Inc., for consulting and participating on advisory boards.

Mairead McGinn was a clinical study investigator and received honoraria and travel support from Alexion Pharmaceuticals, Inc.

William H McAlister was a clinical study investigator and has not received any remuneration from Alexion Pharmaceuticals, Inc.

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Author contributions

Study design: Michael P. Whyte, Scott Moseley

Study investigator: Mairead McGinn, John Taylor, Jill H. Simmons, Nicholas Bishop

Enrolled patients: Mairead McGinn, Jill H. Simmons, Nicholas Bishop

Collection and assembly of data: Mairead McGinn, John Taylor, Jill H. Simmons, Scott Moseley

Data analysis: Scott Moseley

Data interpretation: All authors

Manuscript preparation: Michael P. Whyte

Manuscript review and revisions: All authors

Final approval of manuscript: All authors

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Table 1. TEAEs reported for >25% of patients with HPP treated with asfotase alfa

	Asfotase alfa (N=11) n (%)
Pyrexia	8 (72·7)
Upper respiratory tract infection	8 (72·7)
Craniosynostosis	7 (63·6)
Pneumonia	7 (63·6)
Constipation	6 (54·5)
Otitis media	6 (54·5)
Vomiting	6 (54·5)
Headache	5 (45·5)
Injection site erythema	5 (45·5)
Decreased hemoglobin	4 (36·4)
Dental caries	4 (36·4)
Diarrhea	4 (36·4)
Irritability	4 (36·4)
Nasopharyngitis	4 (36·4)
Pain	4 (36·4)
Pain in extremity	4 (36·4)
Rash	4 (36·4)
Tooth loss	4 (36·4)
Viral infection	4 (36·4)
Acute sinusitis	3 (27·3)
Allergic rhinitis	3 (27·3)

	Asfotase alfa (N=11) n (%)
Decreased oxygen saturation	3 (27·3)
Drug dependence	3 (27·3)
Gastroenteritis	3 (27·3)
Increased urine calcium/creatinine ratio	3 (27·3)
Influenza	3 (27·3)
Injection site hematoma	3 (27·3)
Nausea	3 (27·3)
Papilledema	3 (27·3)
Pharyngitis	3 (27·3)
Procedural pain	3 (27·3)
Respiratory distress	3 (27·3)
Sinusitis	3 (27·3)
Sleep apnea syndrome	3 (27·3)
Tracheitis	3 (27·3)
Wheezing	3 (27·3)

1 TEAE=treatment-emergent adverse event.

2

Figure 1. Sequential application of instruments for assessing motor and cognitive function in infants and children with HPP treated with asfotase alfa. The study site physical therapist chose which to apply based on patient age and functional abilities. Those with severe developmental delay were not transitioned based on age alone. Therefore, for patients older than age 42 months with severe developmental delay, the BSID-III may have continued to be used.

BSID-III=Bayley Scales of Infant and Toddler Development, Third Edition; PDMS-2=Peabody Developmental Motor Scales, Second Edition; BOT-2=Bruininks-Oseretsky Test of Motor Proficiency, Second Edition.

[a]Validated for impaired and healthy infants and toddlers aged 1–42 mo.[24]

[b]Validated for ages ≤ 60 mo.[25]

[c]Validated for ages 48–252 mo.[26]

Figure 2. Median RGI-C scores (A) and RSS scores (B) over time in infants and young children with HPP treated with asfotase alfa. Patients with RGI-C scores of +2 (substantial healing of HPP rickets) or higher (complete healing) were classified as RGI-C "responders."[18] *p<0·05 for change from Baseline based on Wilcoxon signed-rank test. Significant improvement in RGI-C scores was demonstrated by Month 3 of treatment, reaching a median score of +2·0 by Month 6[18] that was sustained ≥+2·0 thereafter. Similarly, significant improvements in RSS scores observed as early as Month 6[18] were sustained at most visits over 7 years of treatment.

HPP=hypophosphatasia; RGI-C=Radiographic Global Impression of Change; RSS=Rickets Severity Scale.

Figure 3. Representative radiographic changes spanning Baseline to Year 6·5 of asfotase alfa treatment are illustrated for (A) the left wrist of patient #1 and (B) the right knee of patient #2. For both patients at Baseline, there are markedly widened physes with indistinct provisional zones of calcification, and metaphyseal flaring (*arrows*), as well as generalized osteopenia consistent with severe rickets. Substantial improvements are apparent at Month 6 of asfotase alfa treatment and sustained at 6·5 years of therapy.

Figure 4. Respiratory support spanning the entire study for infants and young children with HPP treated with asfotase alfa. Although nearly all patients required respiratory support (sometimes prolonged) in the beginning,[18] no support was required by the nine patients beginning mid-Year 4 of treatment[15] and extending to study completion.

CPAP=continuous positive airway pressure; BiPAP=bilevel or biphasic positive airway pressure; HPP=hypophosphatasia.

[a] Noninvasive respiratory support included CPAP, BIPAP, and supplemental oxygen.

[b] Data through Year 6 reported previously for the six patients requiring CPAP, BiPAP, or mechanical ventilation.[15]

Figure 5. Median length/height and weight Z-scores over time in infants and young children with HPP treated with asfotase alfa. Green shaded area reflects the normal range (mean ± 2 SD) for healthy age- and sex-matched peers. *p<0·05 for change from Baseline based on one-sample *t*-test. After Year 3 of treatment, median weight normalized and remained normal through study end despite past failure to thrive for many of the patients. Median length/height also improved but did not reach the normal range.

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HPP=hypophosphatasia.

Figure 6. Scaled BSID-III Gross Motor, Fine Motor, and Cognitive subscale scores (A), PDMS-2 Locomotion subscale standard scores (B), and BOT-2 Running Speed and Agility scaled scores (C) over time in infants and young children with HPP treated with asfotase alfa. The BSID-III was applied for patients aged <43 months. Assessments were performed based on the patient's chronologic age and functional abilities. Green shaded area reflects mean (SD) scaled score for healthy age-matched peers. Stability in scaled scores over time indicates continued acquisition or improvement in quality of motor skills. (**A**) The BSID-III showed improvement in Gross Motor scores by Year 2 of treatment, although scores did not normalize by Last Assessment. BSID-III Fine Motor and Cognitive scores improved by Month 3 and normalized at Year 2. (**B**) Of eight patients completing serial PDMS-2 assessments, seven had standard scores below the normal range at first assessment and 5 achieved normal scores at ~Year 4·5–5·5 of treatment. (**C**) Of eight patients transitioned to the

BOT-2, seven had Running Speed and Agility scores below the normal range at first assessment and three had normal scaled scores at ~Years 6–8.

_ _

BOT-2=Bruininks-Oseretsky Test of Motor Proficiency, Second Edition; BSID-III=Bayley Scales of Infant and Toddler Development, Third Edition; HPP=hypophosphatasia; PDMS-2=Peabody Developmental Motor Scales, Second Edition.

Figure 1

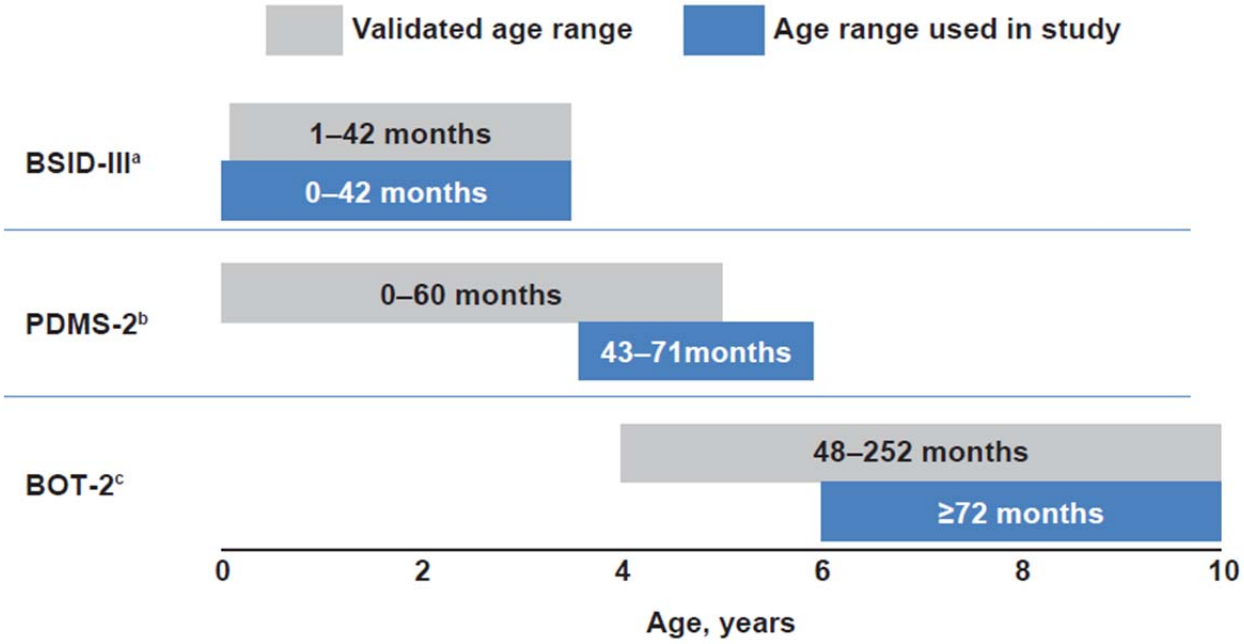

Validated age range
Age range used in study
BSID-III[a]
1–42 months
0–42 months
PDMS-2[b]
0–60 months
43–71months
BOT-2[c]
48–252 months
≥72 months
0
2
4
6
8
10
Age, years

Figure 2A

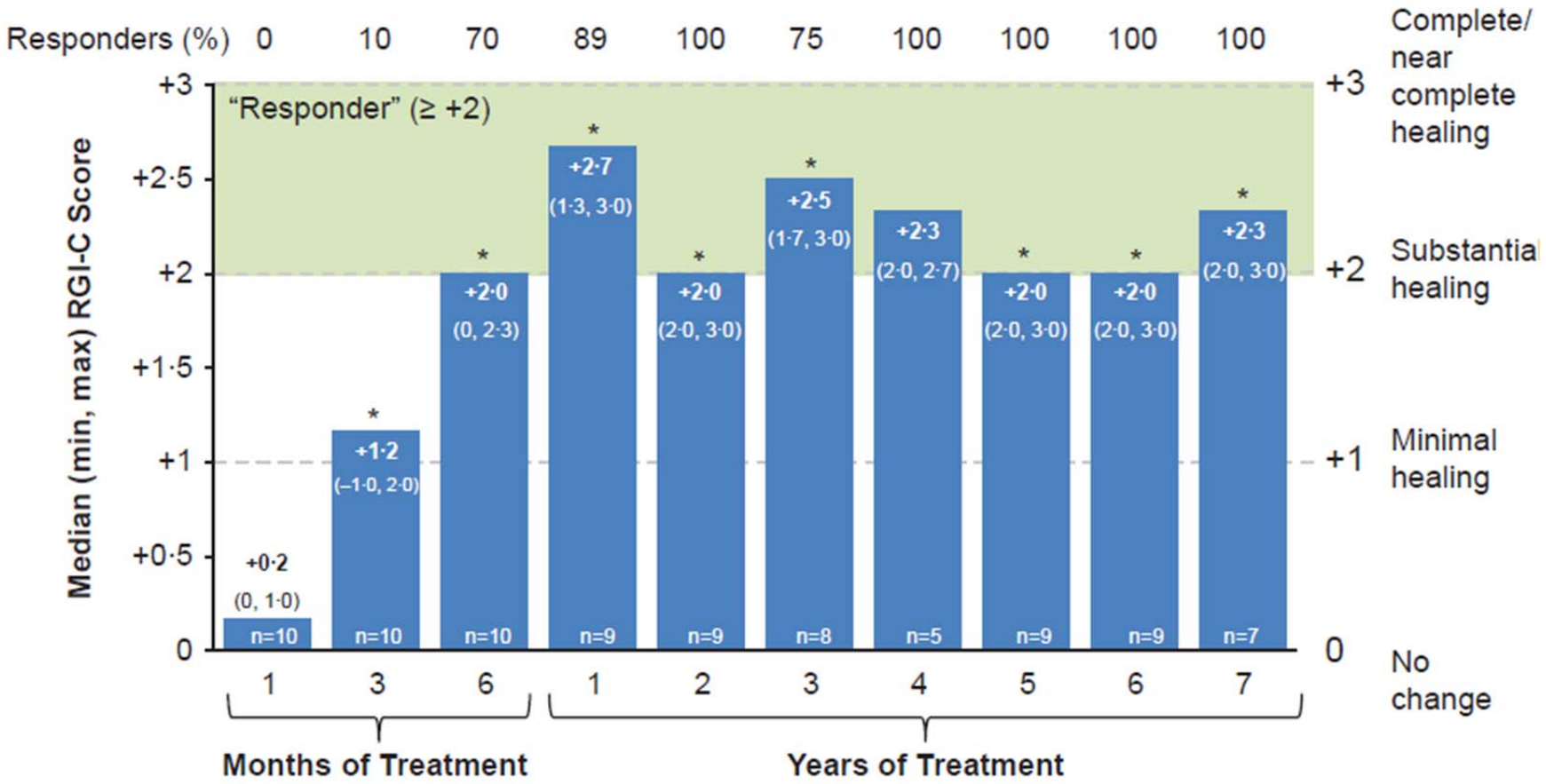

Responders (%) 0 10 70 89 100 75 100 100 100 100
"Responder" (≥ +2)
Median (min, max) RGI-C Score
+3
+2·5
+2
+1·5
+1
+0·5
0
+0·2 (0, 1·0) n=10
+1·2 (–1·0, 2·0) n=10
+2·0 (0, 2·3) n=10
+2·7 (1·3, 3·0) n=9
+2·0 (2·0, 3·0) n=9
+2·5 (1·7, 3·0) n=8
+2·3 (2·0, 2·7) n=5
+2·0 (2·0, 3·0) n=9
+2·0 (2·0, 3·0) n=9
+2·3 (2·0, 3·0) n=7
1 3 6 1 2 3 4 5 6 7
Months of Treatment
Years of Treatment
Complete/ near complete healing
Substantial healing
Minimal healing
No change

Figure 2B

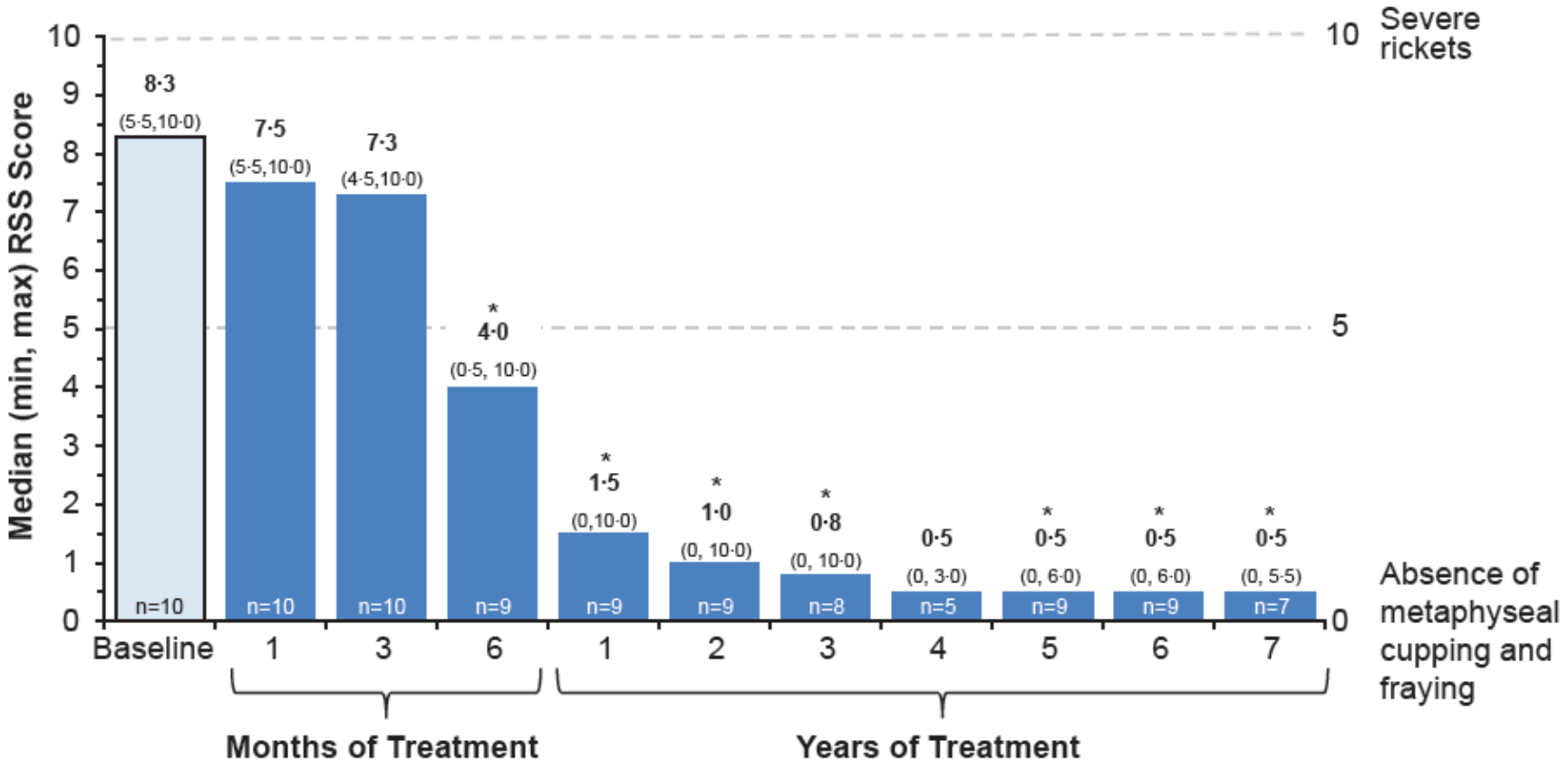

Median (min, max) RSS Score
10
9
8
7
6
5
4
3
2
1
0
10
Severe rickets
5
0
Absence of metaphyseal cupping and fraying
8·3
(5·5,10·0)
n=10
7·5
(5·5,10·0)
n=10
7·3
(4·5,10·0)
n=10
*
4·0
(0·5, 10·0)
n=9
*
1·5
(0,10·0)
n=9
*
1·0
(0, 10·0)
n=9
*
0·8
(0, 10·0)
n=8
0·5
(0, 3·0)
n=5
*
0·5
(0, 6·0)
n=9
*
0·5
(0, 6·0)
n=9
*
0·5
(0, 5·5)
n=7
Baseline
1
3
6
1
2
3
4
5
6
7
Months of Treatment
Years of Treatment

Figure 3A

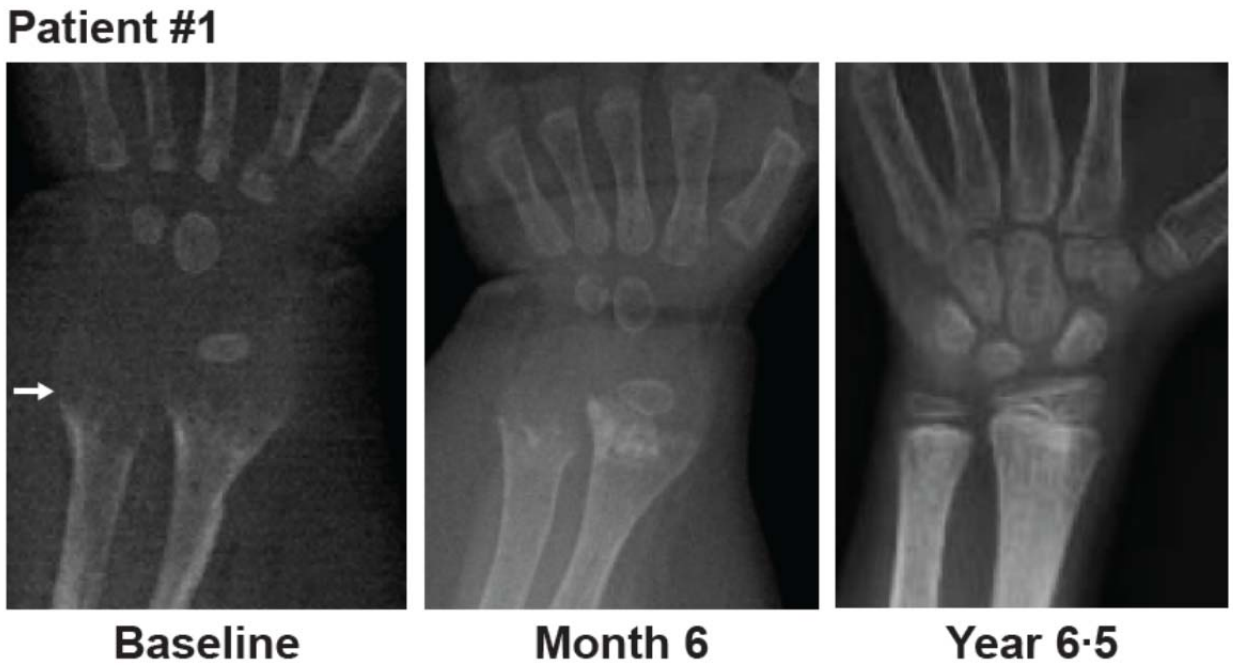


Figure 3B

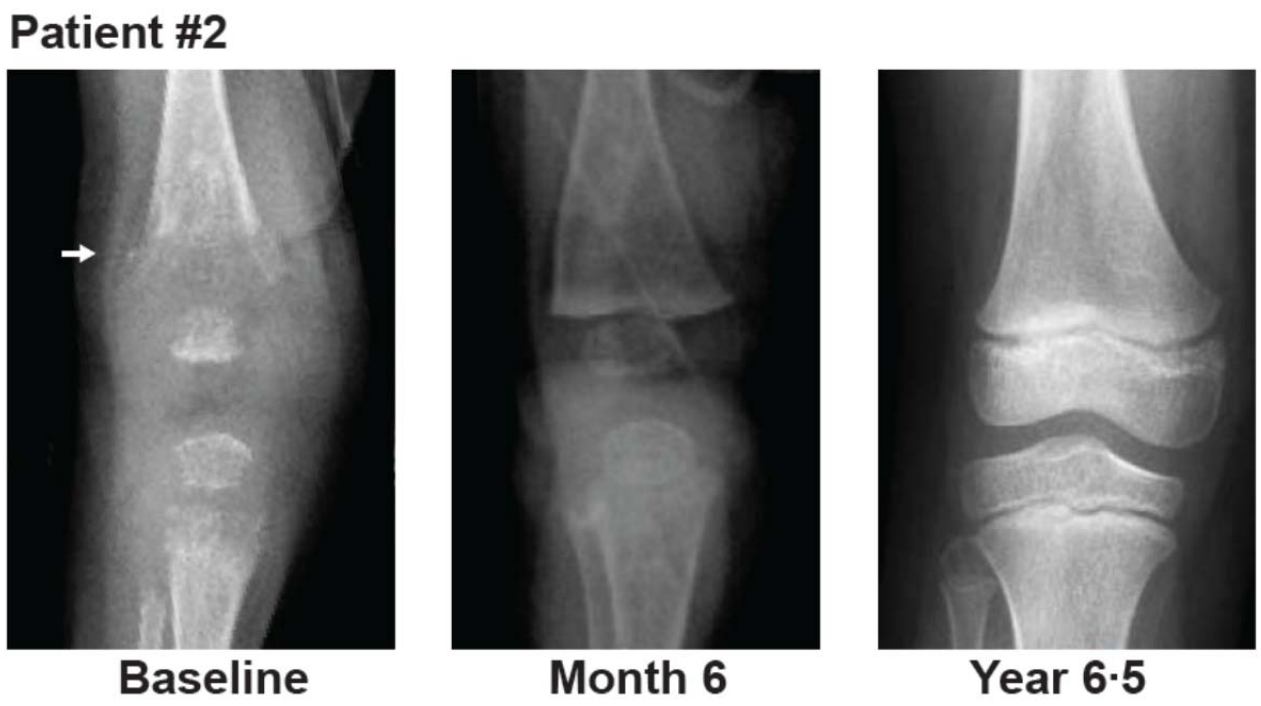


Figure 4

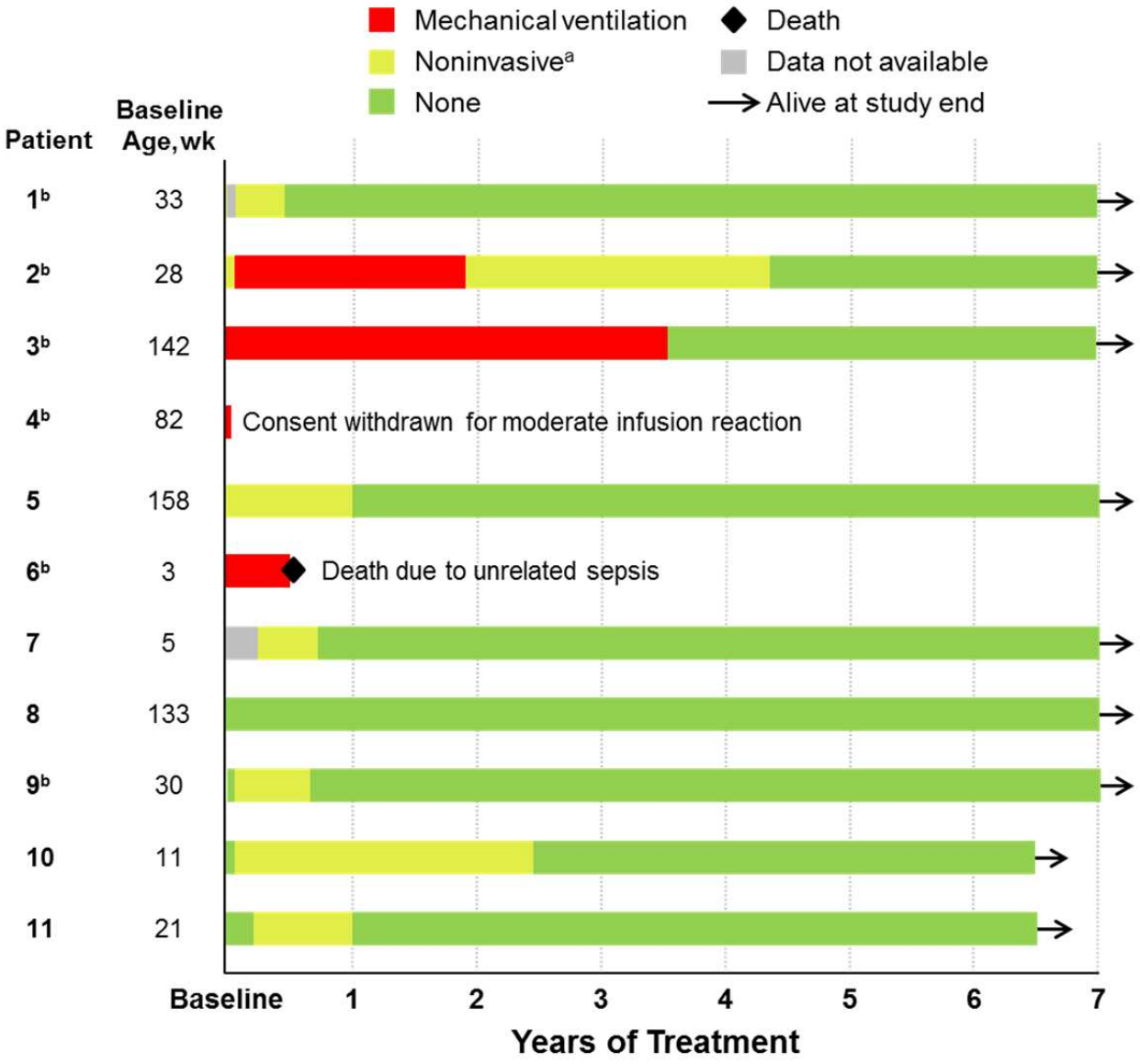
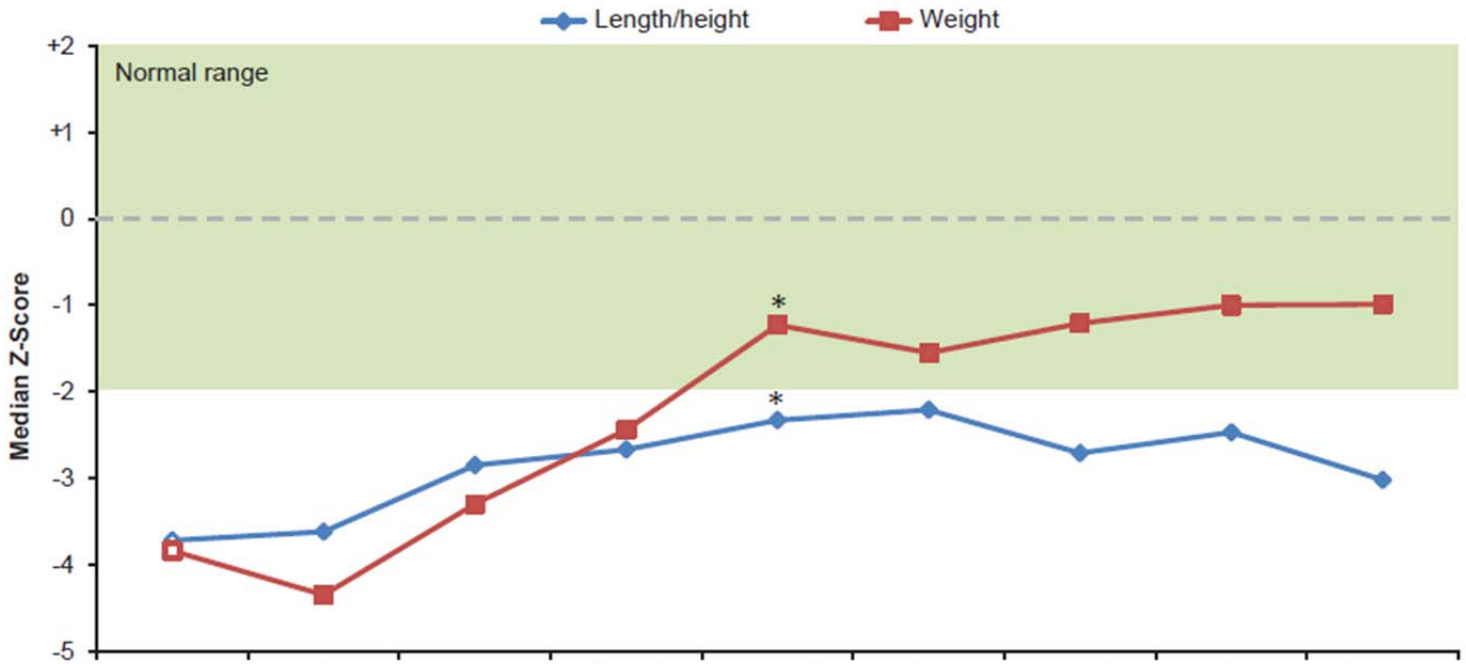

Mechanical ventilation
Noninvasive[a]
None
Death
Data not available
Alive at study end
Patient
Baseline Age, wk
1[b] 33
2[b] 28
3[b] 142
4[b] 82
Consent withdrawn for moderate infusion reaction
5 158
6[b] 3
Death due to unrelated sepsis
7 5
8 133
9[b] 30
10 11
11 21
Baseline
1
2
3
4
5
6
7
Years of Treatment

Figure 5



	Baseline n=11	Month 6 n=10	Year 1 n=9	Year 2 n=9	Year 3 n=8	Year 4 n=6	Year 5 n=9	Year 6 n=9	Year 7 n=7
Length/height Z-score									
Median (min, max)	−3·72 (−9·2, −0·7)	−3·62 (−8·2, −1·8)	−2·85 (−9·2, −1·2)	−2·67 (−8·4, −1·0)	−2·33 (−8·6, −0·4)	−2·21 (−5·0, +0·3)	−2·71 (−9·0, +0·1)	−2·47 (−8·6, −0·5)	−3·02 (−8·7, −0·6)
Change from Baseline, Mean (95% CI)		+0·18 (−0·60, +0·97)	+0·62 (−0·27, +1·52)	+1·00 (−0·43, +2·42)	+1·69 (+0·12, +3·25)	+1·46 (−1·98, +4·90)	+1·24 (−0·79, +3·26)	+1·37 (−0·44, +3·18)	+0·55 (−1·62, +2·73)
Weight Z-score									
Median (min, max)	−3·84 (−5·4, −0·5)	−4·35 (−6·4, −1·5)	−3·30 (−6·3, −1·7)	−2·44 (−4·8, −0·9)	−1·23 (−5·1, 0·4)	−1·55 (−3·6, −0·8)	−1·21 (−5·0, +0·2)	−1·00 (−5·6, −0·1)	−0·99 (−3·7, +0·5)
Change from Baseline, Mean (95% CI)		−0·53 (−1·36, +0·29)	+0·32 (−0·98, +1·61)	+1·03 (−0·63,+ 2·69)	+2·43 (+0·80, +4·06)	+1·27 (−1·17, +3·72)	+1·85 (−0·17, +3·86)	+2·02 (−0·10, +4·14)	+2·40 (−0·31, +5·10)

Figure 6A

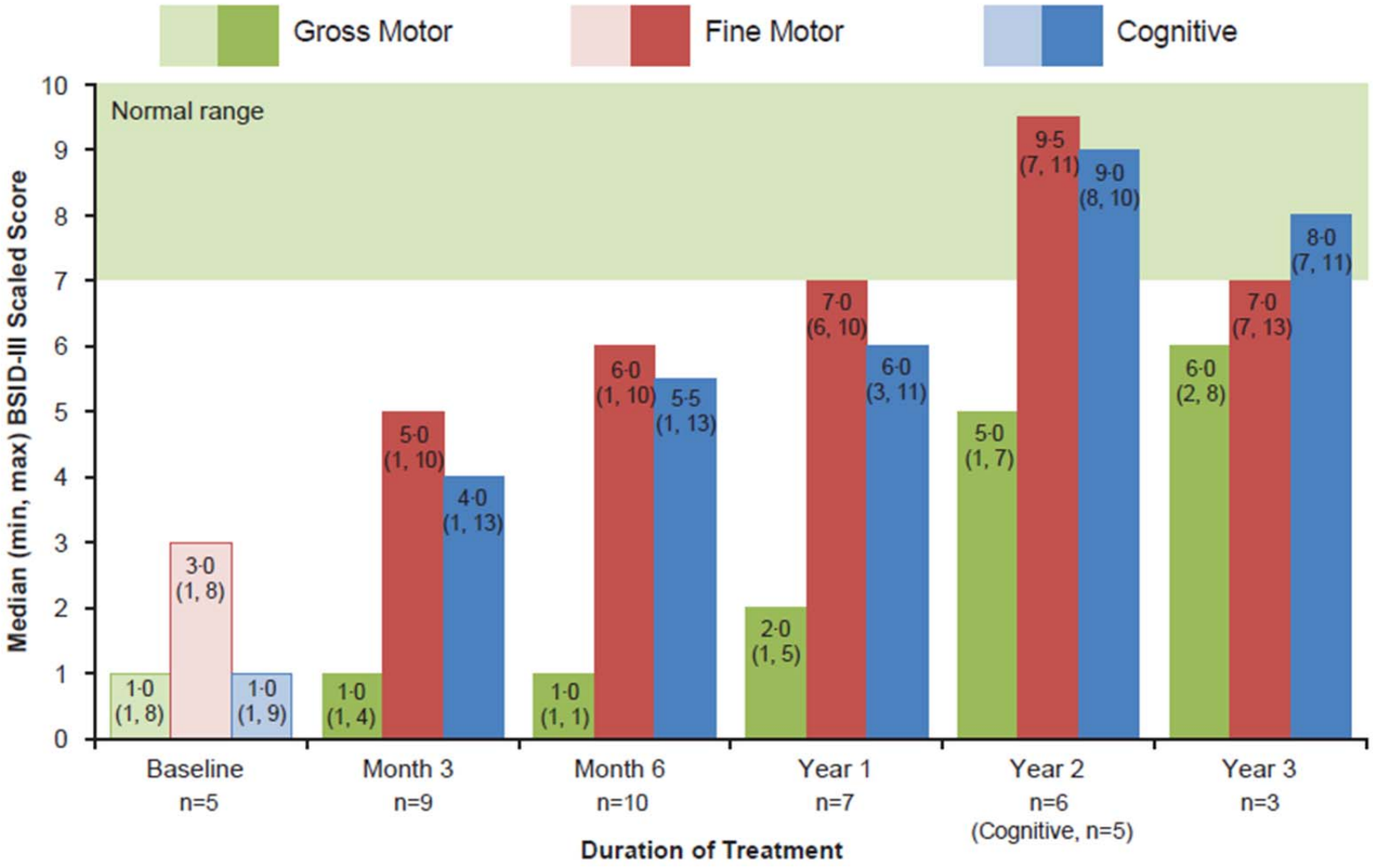

Gross Motor
Fine Motor
Cognitive
Normal range
Median (min, max) BSID-III Scaled Score
10
9
8
7
6
5
4
3
2
1
0
1·0 (1, 8)
3·0 (1, 8)
1·0 (1, 9)
1·0 (1, 4)
5·0 (1, 10)
4·0 (1, 13)
1·0 (1, 1)
6·0 (1, 10)
5·5 (1, 13)
2·0 (1, 5)
7·0 (6, 10)
6·0 (3, 11)
5·0 (1, 7)
9·5 (7, 11)
9·0 (8, 10)
6·0 (2, 8)
7·0 (7, 13)
8·0 (7, 11)
Baseline n=5
Month 3 n=9
Month 6 n=10
Year 1 n=7
Year 2 n=6 (Cognitive, n=5)
Year 3 n=3
Duration of Treatment

Figure 6B

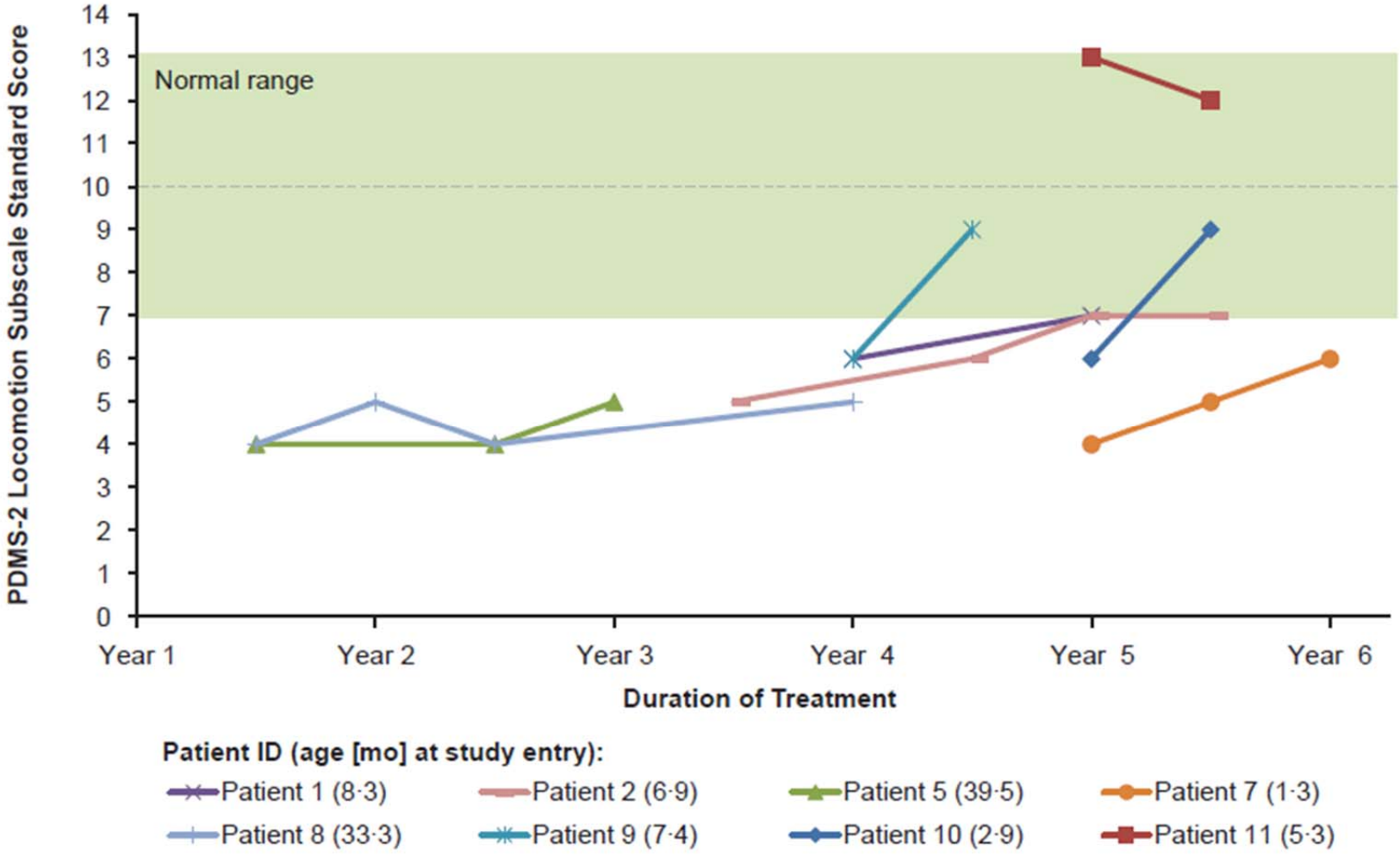
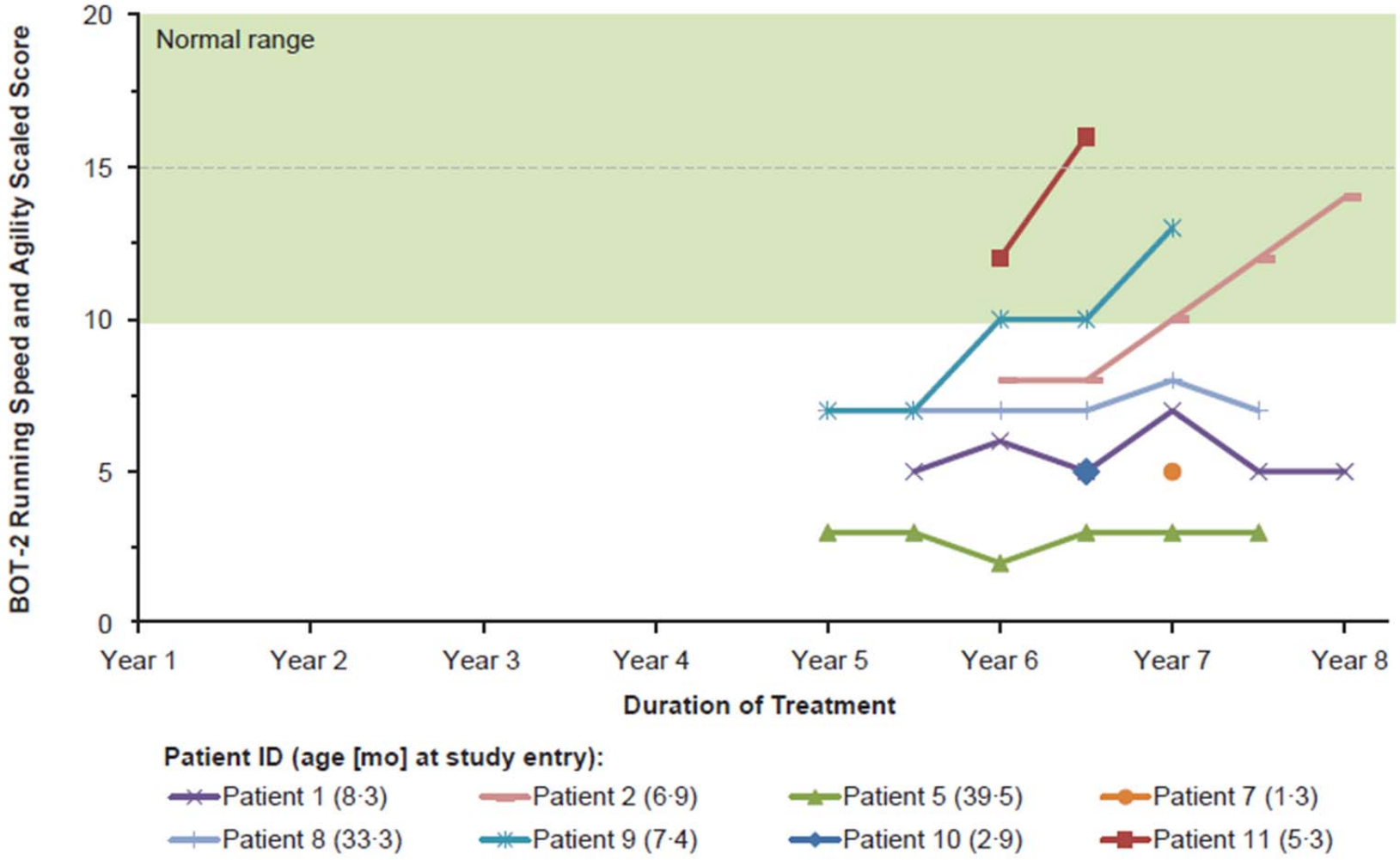

PDMS-2 Locomotion Subscale Standard Score
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Normal range
Year 1
Year 2
Year 3
Year 4
Year 5
Year 6
Duration of Treatment
Patient ID (age [mo] at study entry):
Patient 1 (8·3)
Patient 2 (6·9)
Patient 5 (39·5)
Patient 7 (1·3)
Patient 8 (33·3)
Patient 9 (7·4)
Patient 10 (2·9)
Patient 11 (5·3)

Figure 6C


Normal range
BOT-2 Running Speed and Agility Scaled Score
20
15
10
5
0
Year 1
Year 2
Year 3
Year 4
Year 5
Year 6
Year 7
Year 8
Duration of Treatment
Patient ID (age [mo] at study entry):
Patient 1 (8·3)
Patient 2 (6·9)
Patient 5 (39·5)
Patient 7 (1·3)
Patient 8 (33·3)
Patient 9 (7·4)
Patient 10 (2·9)
Patient 11 (5·3)