Asfotase alfa for infants and young children with hypophosphatasia: 7 year outcomes of a single-arm, open-label, phase 2 extension trial

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Summary

Background Our previous phase 2, open-label study of 11 infants and young children with life-threatening perinatal or infantile hypophosphatasia showed 1 year safety and efficacy of asfotase alfa, an enzyme replacement therapy. We aimed to report the long-term outcomes over approximately 7 years of treatment.

Methods We did a prespecified, end of study, 7 year follow-up of our single-arm, open-label, phase 2 trial in which children aged 3 years or younger with life-threatening perinatal or infantile hypophosphatasia were recruited from ten hospitals (six in the USA, two in the UK, one in Canada, and one in the United Arab Emirates). Patients received asfotase alfa (1 mg/kg three times per week subcutaneously, adjusted to 3 mg/kg three times per week if required) for up to 7 years (primary treatment period plus extension phase) or until the product became commercially available; dosage adjustments were made at each visit according to changes in the patient’s weight. The primary objectives of this extension study were to assess the long-term tolerability of asfotase alfa, defined as the number of patients with one or more treatment-emergent adverse events, and skeletal manifestations associated with hypophosphatasia, evaluated using the Radiographic Global Impression of Change (RGI-C) scale (−3 indicating severe worsening, and +3 complete or near-complete healing). Respiratory support, growth, and cognitive and motor functions were also evaluated. All efficacy and safety analyses were done in all patients who received any asfotase alfa (full-analysis population). This study and extension phase are registered with ClinicalTrials.gov, number NCT01205152, and EudraCT, number 2009-009369-32.

Findings 11 participants were recruited between Oct 6, 2008, and Dec 4, 2009. Ten patients completed a 6 month treatment period and entered the extension phase; nine received asfotase alfa for at least 6 years and completed the study, with four being treated for more than 7 years. Skeletal healing was sustained over 7 years of treatment; all evaluable patients had RGI-C scores of at least +2 at year 6 (n=9; median score +2·0 [range 2·0–3·0]) and year 7 (n=7; median score +2·3 [2·0–3·0]). 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 or 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. All 11 patients had at least one treatment-emergent adverse event. The most common adverse events were pyrexia (eight [73%] of 11 patients), upper respiratory tract infection (eight [73%]), craniosynostosis (seven [64%]), and pneumonia (seven [64%]). Serious adverse events related to asfotase alfa occurred in three (27%) patients (severe chronic hepatitis; moderate immediate post-injection reaction; and severe craniosynostosis with severe conductive deafness).

Interpretation Patients with perinatal or infantile hypophosphatasia treated with asfotase alfa for up to 7 years showed early, sustained improvements in skeletal mineralisation. Respiratory function, growth, and cognitive and motor function also improved, and asfotase alfa was generally well tolerated.

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Introduction

Hypophosphatasia is the rare, inherited, metabolic bone disease caused by loss-of-function mutations of the alkaline phosphatase biomineralisation-associated gene (ALPL) that encodes the tissue non-specific isoenzyme of alkaline phosphatase (TNSALP). Low TNSALP activity on cell surfaces results in extracellular accumulation of its substrates, including inorganic pyrophosphate (PPi) and pyridoxal 5’-phosphate (PLP).1,2,5,6 PPi potently inhibits mineralisation by blocking hydroxyapatite crystal formation.6,7 Thus, the superabundance of PPi in hypophosphatasia often leads to rickets during growth.1,3 TNSALP dephosphorylates PLP (the principal circulating form of vitamin B6) to pyridoxal, which allows the compound to cross cell plasma membranes and be rephosphorylated...
Research in context

Evidence before this study
In 2012, we reported the 1 year findings from the first open-label, phase 2, multinational study that evaluated the safety and efficacy of asfotase alfa, an enzyme replacement therapy, in infants and young children with the life-threatening perinatal or infantile forms of hypophosphatasia, a heritable metabolic bone disease. Clinically significant healing of the skeleton was accompanied by improved respiratory function and developmental milestones, and this drug was generally well tolerated. At that time, no treatment was approved for hypophosphatasia, and management of the disease involved supportive care. Asfotase alfa was approved multinational in 2015, based in part on the findings from this pivotal open-label study. In a 2016 publication, these same patients had improved survival and respiratory outcomes compared with historical controls. In another 2016 study, older children with symptomatic hypophosphatasia showed sustained improvement in skeletal mineralisation, with most achieving normal values for age-matched and sex-matched peers in growth, strength, and motor function during 5 years of treatment with asfotase alfa.

Added value of this study
This Article reports long-term follow-up data since the initial 2012 publication. The impact of asfotase alfa treatment is presented for infants and young children with life-threatening hypophosphatasia who were given a median of 6·6 years of therapy, representing the longest follow-up to date in patients with hypophosphatasia receiving treatment with asfotase alfa. The early improvements were sustained for up to 7 years of treatment. Typically, the improved skeletal mineralisation during the first 6 months of treatment was followed by withdrawal of respiratory support, and was then associated with improved motor and cognitive function that persisted 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 or height and weight Z scores indicated catch-up growth. Improvements from baseline in gross motor, fine motor, and cognitive function reached levels that could match those of 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 the treatment of older children with hypophosphatasia. For life-threatening paediatric-onset hypophosphatasia, prompt diagnosis and commencement of asfotase alfa treatment can rescue such patients and give them enjoyable health.

in intracellularly to PLP. Therefore, vitamin B6-dependent seizures occur in some infants who are severely affected by hypophosphatasia. Life-threatening complications in the severe perinatal and infantile forms of hypophosphatasia can include respiratory failure from rachitic chest deformity and rib fractures, elevated intracranial pressure due to craniosynostosis, and hypercalcaemia leading to nephrocalcinosis and renal compromise. Other potential complications of paediatric hypophosphatasia include long-bone deformity and muscle weakness. Historically, perinatal hypophosphatasia has been considered lethal, and infantile hypophosphatasia has around 50% mortality during the first year of life. Asfotase alfa (Strensiq, Alexion Pharmaceuticals, Boston, MA, USA) is a human, recombinant, TNSALP replacement therapy that was approved multinational in 2015, typically for the treatment of paediatric-onset hypophosphatasia. The safety and efficacy of asfotase alfa were first evaluated during our phase 2, open-label study in paediatric patients aged 3 years or younger with life-threatening perinatal or infantile hypophosphatasia. This study enrolled 11 patients with hypophosphatasia (five with perinatal hypophosphatasia and six with infantile hypophosphatasia) ranging in age from 2 weeks to 3 years for the 6 month initial trial. Patients manifested complications of hypophosphatasia before age 6 months, including skeletal abnormalities, such as shortened or bowed limbs, rachitic chest deformity, fractures, osteopenia, craniosynostosis, or other rachitic findings. All but one patient had failure to thrive, most patients (nine [82%] of 11) required respiratory support, and all had gross motor delay. A single 2 mg/kg intravenous infusion of asfotase alfa preceded subcutaneous injections, starting at 1 mg/kg three times per week. Results from this study published in 2012 showed outcomes after 12 months or longer (range 12–26) of treatment with asfotase alfa. One patient had consent withdrawn on day 1 because of infusion-associated reactions, and one patient died of pneumonia and sepsis after 7-5 months of treatment. The study met its primary efficacy measure of change in hypophosphatasia skeletal disease severity from baseline to month 6 on the basis of assessment of skeletal radiographs using the validated 7 point Radiographic Global Impression of Change (RGI-C) scale (median +2·0, minimum 0, maximum +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. In this Article, 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 and participants
In this Article, we present the prespecified 7 year follow-up of our single-arm, open-label, phase 2 trial. The trial design, including patient inclusion and exclusion criteria, has been published elsewhere.18 The patient numbering scheme used in our previous publication is continued in this report. Briefly, this study was done at ten hospitals (six sites in the USA, two in the UK, one in Canada, and one in the United Arab Emirates). Key eligibility criteria were age 3 years or younger, and a documented diagnosis of severe hypophosphatasia with symptoms occurring before the age of 6 months.18

The investigation was authorised at each study site through appropriate research governance and ethics processes. Parents and legal guardians signed the written consent form before study participation.

Procedures
In the 6 month primary treatment period, safety and efficacy of asfotase alfa were assessed. A single 2 mg/kg intravenous infusion of asfotase alfa preceded 1 mg/kg subcutaneous injections of asfotase alfa three times per week (total dose 3 mg/kg per week). The dosage could be increased to up to 3 mg/kg three times per week (total dose 9 mg/kg per week) after 1 month if the treatment was not effective, defined as worsening of failure to thrive, deteriorating pulmonary function, or no radiographic evidence of skeletal improvement. The extension phase continued the subcutaneous asfotase alfa dosage from the primary treatment period; dosage adjustments were made at each visit according to changes in the patient’s weight. Additional dosage adjustments (no limits on maximum dose) were permitted if the treatment was not effective or for safety-related concerns. Patients continued to receive asfotase alfa for up to 7 years (primary treatment period plus extension phase) or until the product became commercially available, whichever occurred first.

Hypophosphatasia 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, 2, 5, 7, 3, 4, 5, 6, and 7. At each timepoint, the same three paediatric radiologists independently rated changes compared with baseline using the RGI-C scale (−3 indicating severe worsening, 0 no change, and +3 complete or near-complete healing), which has been validated in paediatric patients with hypophosphatasia.26 The mean of the RGI-C scores was calculated for each patient at each timepoint. A separate rater independently evaluated the radiographs of the wrists and knees at each timepoint using the 10 point Rickets Severity Scale (RSS; from 0, indicating absence of metaphyseal cupping and fraying, to 10, indicating 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 individuals who rated RGI-C and RSS were masked to all treatment timepoints (except baseline radiographs for RGI-C) and all other patient information.

Use of supplemental oxygen, continuous positive airway pressure (CPAP), bi(biphase) positive airway pressure (BiPAP), and mechanical ventilation was documented at each study visit.

Length or height, weight, and head circumference were recorded at study visits. Z scores for length or height and weight were established using US Centers for Disease Control and Prevention growth charts for age-matched and sex-matched healthy infants and children.22 Head circumference Z scores were calculated using WHO formulae.23 Depending on the patient’s age and functional abilities at individual visits, each site’s physical therapist, in consultation with the medical monitor, established the appropriate assessment or combination of assessments of motor and cognitive development, which included the following: 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; appendix). Patients aged 42 months or younger, or those who were older but with severe developmental delays, were assessed using the gross motor, fine motor, and cognitive subscales of the BSID-III.24 If patients showed cognitive age equivalence of 42 months, such testing was discontinued. Patients aged 43–71 months who were considered to have evaluable functional abilities were studied using the locomotion subtest of the PDMS-2, an assessment of gross motor skills.25 Patients aged 72 months or older with evaluable functional abilities completed the running speed, agility, and strength subtests of the BOT-2, an assessment of gross motor proficiency.26 Licensed physical therapists, or their local equivalents, did the assessments at baseline (BSID-III only), month 3 (BSID-III only), month 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.

Blood was collected for assay of serum alkaline phosphatase (ALP) activity, plasma PPI and PLP concentrations, and serum intact parathyroid hormone at baseline, month 3, month 6, month 9, year 1, and every 6 months thereafter. Treatment samples were collected before asfotase alfa dosing and after patients had fasted for at least 4 h. The tubes for blood sampling of PPI and PLP contained leva(misole) to inhibit the high ALP activity from asfotase alfa. Laboratory samples were managed by a central facility (Covance, Indianapolis, USA).
IN, USA, and Geneva, Switzerland). PPI analyses were done by Alexion Montreal Corporation (Montreal, QC, Canada) and Charles River Laboratories (Senneville, QC, Canada). PLP analyses were done at two central laboratories (ARUP Laboratories, Salt Lake City, UT, USA, and Biotrial Bioanalytical Services, Laval, QC, Canada). Reported PLP results were not censored for vitamin B6 supplementation, which can markedly increase PLP in patients with hypophosphatasia. Additional details regarding the PPI and PLP assays are provided in the appendix.

Adverse events, including injection site reactions (ISRs) and injection-associated reactions (IARs), were continuously monitored. ISRs were defined as adverse events localised to the site of asfotase alfa administration, and IARs were defined as systemic signs, symptoms, or findings (eg, chills, cough, and erythema) occurring within 3 h after asfotase alfa administration. The site investigator assessed the possible, probable, or definite relationship of adverse events to the study drug. Additional safety assessments included physical examination findings, laboratory values (including calcium and phosphate), and asfotase alfa antibody testing results (done by Cambridge Biomedical, Boston, MA, USA, and PPD Laboratories, Richmond, VA, USA). Patients were assessed for ectopic calcification by periodic funduscopic examinations (changed to full ophthalmological examinations by protocol amendment after about 1.5 years) and periodic renal ultrasounds.

Outcomes

In this extension trial, the primary safety outcome was the long-term tolerability of asfotase alfa, defined as the number of patients with one or more treatment-emergent adverse events. The primary efficacy outcome was long-term skeletal manifestations of hypophosphatasia, as measured using the RGI-C scale as previously described. Secondary outcome measures were long-term pharmacodynamics of asfotase alfa, the effect of asfotase alfa on growth and development, survival, respiratory function, and other clinical signs and symptoms of hypophosphatasia in infants and young children.

Statistical analyses

As published elsewhere, the sample size for this study reflected findings from a previous study showing the correlation of a 10-point RSS with severity of nutritional rickets, with variability of approximately 0.75 points. Assuming similar variability in a population of patients with hypophosphatasia and a sample size of six patients, pretreatment versus post-treatment radiographic differences must be at least 1.9 points for 80% power to detect a significant difference. This number was increased to ten patients to establish changes after 24 weeks of treatment. All efficacy and safety analyses were done using the full-analysis population, which included all patients who received any asfotase alfa. For the purposes of this study, the full-analysis population and intent-to-treat population were identical. Analyses of the secondary endpoints will include all patients with available data at each timepoint. Some analyses were repeated using the per-protocol 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 timepoints were approximated, with 48 weeks defined as 1 year.

Median (minimum, maximum) values were calculated for the RGI-C and RSS scores, length or height, weight, and head circumference raw values and Z scores, and BSID-III scaled scores over time. A one-sample Wilcoxon signed-rank test using a two-sided α of 0.05 was used to test whether the median RGI-C score at each timepoint differed from 0 (ie, no change). The proportion of patients with RGI-C scores of +2 or +3 (so-called RGI-C responders) was calculated for each timepoint. Mean changes from baseline in Z scores for length or height and weight were analysed using a one sample t test. Mean and scaled (or standard) scores were calculated for each subscale or subtest of the BSID-III, PDMS-2, and BOT-2. Scaled or standard scores for each subscale or subtest were then compared with the normative mean and SD values for healthy age-matched peers, which were 10 (SD 3) for the BSID-III scaled score, 10 (3) for the PDMS-2 standard score, and 15 (5) for the BOT-2 running speed and agility subtest scaled score. These functional measures were exploratory and therefore not analysed statistically.

Pharmacodynamic and safety outcome measures are summarised descriptively, SAS version 9.4 (Cary, NC, USA) was used for all statistical analyses.

This extension trial is registered with ClinicalTrials.gov, number NCT01205152, and EudraCT, number 2009-009369-32. The study was overseen by a data monitoring committee.

Role of the funding source

The funder was involved in all stages of the study conduct and analysis, including the study design, data collection, data analysis, data interpretation, and writing of this report. The corresponding author had full access to all the data in the study and had final responsibility to submit for publication.

Results

11 patients were enrolled between Oct 6, 2008, and Dec 4, 2009, and received at least one dose of asfotase alfa (median age at enrolment 30 weeks [range 3–158; seven [64%] girls and four [36%] boys). All ten patients who completed the 6 month primary treatment period entered the extension phase; nine patients received...
asfotase alfa for at least 6 years and completed the study, with four of the nine patients being treated for more than 7 years. The median duration of treatment for the 11 enrolled patients was 6·6 years (range 1 day to 7·5 years). Patient demographics, baseline characteristics, and efficacy and safety outcomes after at least 1 year of treatment for the nine patients who were treated for at least 6 years in the current trial and detailed case reports of all 11 enrolled patients have been published elsewhere. Updated narratives for all patients who completed the study are provided in the appendix.

Four patients had major protocol deviations considered to potentially influence treatment effect and were therefore excluded from the per-protocol population. Two patients did not meet the ALP eligibility criterion (ALP activity at least 3 SDs below the mean ALP activity for that age). One patient did not meet the failure-to-thrive eligibility criterion (participant was too young developmentally for assessment) and received a fixed dose of asfotase alfa that was not adjusted to weight (received subcutaneous injections of 8 mg asfotase alfa three times per week) from approximately week 9 until his death at 7·5 months, 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 three times per week to 1·5 mg/kg [one dose only]) and day 24 (from 1·5 mg/kg to 3 mg/kg three times per week); dose increases were not permitted per protocol before completion of 1 month of treatment. Because primary efficacy outcomes (RGI-C scores at month 6) were similar between the full-analysis and

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**Figure 1:** Median RGI-C scores and RSS scores over time in infants and young children with hypophosphatasia treated with asfotase alfa

(A) Median (range) in RGI-C scores across the treatment period. An RGI-C score of +2 (substantial healing of hypophosphatasia rickets) or higher (complete healing) was classified as RGI-C responders. Updated narratives for all patients who completed the study are provided in the appendix. Because primary efficacy outcomes (RGI-C scores at month 6) were similar between the full-analysis and
per-protocol populations (data not shown), we present results after 6–7 years of asfotase alfa treatment for the full-analysis population only.

Median RGI-C scores documented improvements in hypophosphatasia skeletal disease as early as month 3, which were typically sustained over 7 years of treatment, with significant (p<0.05) improvements at most visits (figure 1A). The proportion of evaluable patients with RGI-C scores of at least +2 (responders) was eight (89%) of nine patients at year 1 and seven (100%) of seven patients at year 7. The highest possible RGI-C score of +3 was achieved by four patients, with three first achieving a +3 score by year 1 and one first achieving a +3 by year 2; all four patients maintained scores of at least +2 at all timepoints thereafter.

Median RSS scores indicated that the improvements documented as early as month 6 were sustained over 7 years of treatment, with significant (p<0.05) decreases indicating improvement from baseline at most visits (figure 1B).

All ten patients who entered the extension phase had an overall RGI-C score at last assessment that ranged from +2 (substantial healing) to +3 (complete or near-complete healing). The substantial improvements in radiographic findings for the wrists and knees in two patients treated with asfotase alfa over 7 years of treatment are illustrated in the representative images in figure 2. Illustrative radiographs for all patients are included in the appendix.

Duration of respiratory support was published in 2016.15 for the six patients from this study requiring

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**Figure 2: Representative radiographic changes from baseline to year 6.5 in two patients treated with asfotase alfa**

Radiographic changes are illustrated for the left wrist of patient 1 (A) and the right knee of patient 2 (B). For both patients at baseline, markedly widened physes with indistinct provisional zones of calcification, metaphyseal flaring (arrows), and generalised osteopenia consistent with severe rickets can be observed. Substantial improvements are apparent at month 6 of asfotase alfa treatment, and these improvements are sustained after 6.5 years of therapy.
CPAP, BiPAP, or mechanical ventilation, with a maximum follow-up of 6 years. Respiratory support over time is summarised for all 11 patients (figure 3). At baseline, five (45%) of 11 patients required respiratory support, with three (27%) of 11 requiring mechanical ventilation, one (9%) of 11 receiving CPAP, and one (9%) of 11 receiving supplemental oxygen. By year 2, three (33%) of nine patients required respiratory support, with one (11%) of nine requiring mechanical ventilation, and two (22%) of nine receiving just supplemental oxygen. From 4·5 years of treatment until study end, none of the nine patients required respiratory support (including supplemental oxygen).

Median length or height was 56·5 cm (range 39·0–83·0) at baseline (n=11) and 112·5 cm (88·1–123·0) at year 7 (n=7). The median length or height Z score was higher than at baseline from month 6 until year 7, although this value remained more than 2 SDs below the mean for healthy age-matched and sex-matched peers at all timepoints (figure 4). Overall, four (44%) of nine patients had Z scores within the normal range at last assessment. The mean increase from baseline in length or height Z score was statistically significant at year 3 (p=0·0385) and year 4·5 (p=0·0346), but not at other timepoints.

Median weight was 4·1 kg (range 2·1–9·2) at baseline (n=11) and 19·8 kg (range 15·1–31·4) at year 7 (n=7). Median weight Z scores increased to within 2 SD of the mean for healthy age-matched and sex-matched peers at most timepoints from year 3 to year 7 (figure 4). 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 timepoints.

Median head circumference was 41·5 cm (range 33·0–47·6) at baseline (n=11) and 50·5 cm (44·5–51·3) at year 7 (n=7). Head circumference Z scores remained stable, with a median value of −1·01 (range −4·0 to 0·8) at baseline (n=11) and −1·34 (−4·1 to 1·0) at last assessment (n=10; WHO criteria allow for calculation of head circumference Z scores for patients aged up to 5 years).

At baseline or first assessment, nine (82%) of 11 patients had BSID-III gross motor scaled scores of 1 (3 SDs below the normative mean). Nine (82%) of 11 patients had serial BSID-III assessments (two patients had one assessment each because of discontinuation and death; appendix). All nine patients showed improvements in age-equivalent scores on the gross motor, fine motor, and cognitive subscales (appendix). Median scaled gross motor scores improved from 1 (range 1–8) at baseline to 6 (2–8) at year 3 (normative mean 10, SD 3; figure 5A), indicating motor skill improvement and less developmental delay. Median scores on the fine motor and cognitive subscales were low at baseline but normalised at year 2 and year 3 (figure 5A).

Eight (73%) of 11 patients advanced to complete serial PDMS-2 assessments (appendix). All eight patients showed continued motor skill acquisition on the PDMS-2 locomotion subtest (ie, increased age-equivalent scores; appendix). Among them, seven (88%) of eight patients had standard scores more than 1 SD below the normal reference range (score <7) when they first completed the assessment; five (63%) of eight patients achieved scores within 1 SD of normal (figure 5B).

Eight (73%) of 11 patients transitioned to the BOT-2 and completed at least one BOT-2 assessment (appendix). All had received asfotase alfa for a least 5 years when first tested. Seven (88%) of eight patients had initial scaled BOT-2 running speed and agility subtest scores more than 1 SD below normal (scaled score <10; figure 5C). Three achieved normal scaled scores (>10) by the end of the study. All six patients who completed serial BOT-2 assessments had improved age-equivalent BOT-2 scores during treatment (appendix).

Baseline serum ALP activity was low (median 21 U/L [range 9–46]) in the nine evaluable patients, and increased by more than 100 times by day 2 after the single intravenous infusion of asfotase alfa (median 2990 U/L [range 449–8007]; n=9), and remained elevated thereafter with subcutaneous dosing (eg, 5304 U/L [1812–10 085] at year 1 [n=8] and 4143 U/L [2267–6792] at year 7 [n=7]).

Baseline plasma concentrations of PPI were elevated in four (50%) of eight evaluable patients (median 5·2 μM [range 2·9–10·5]; normal range for patients aged 0–12 years 1·33–5·71 μM; appendix). Median PPI concentrations decreased for all nine patients tested at month 3 of treatment (2·6 μM [range 1·0–4·4]), and remained decreased relative to baseline at other study times.
this patient was not receiving vitamin B6 supplementation any time during the study (range 81.3–960 ng/mL); the course of treatment is shown in the appendix. Overall, the data appeared similar when patients receiving vitamin B6 supplementation were excluded from the analysis (data not shown). One patient’s PLP concentrations failed to normalise at one visit including year 7 (4.6 μM [2.1–10.2]; n=7), with the exception of isolated fluctuations for individual patients.

Baseline plasma concentrations of PLP were elevated in all nine evaluable patients (median 421.0 ng/mL [range 100.0–880.0]; normal range for patients aged 0–5 years 11.8–68.4 ng/mL; appendix). Median PLP concentrations stayed within the normal range from month 6 (47.6 ng/mL [16.4–1510.0]; n=10) to year 7 (38.8 ng/mL [19.1–161.0]; n=7; normal range for patients aged 5–18 years 5.7–61.2 ng/mL). Only one patient’s PLP concentrations failed to normalise at any time during the study (range 81.3–960 ng/mL); this patient was not receiving vitamin B6 supplementation. Overall, the data appeared similar when patients receiving vitamin B6 supplementation were excluded from the analysis (data not shown).

Serum concentrations of parathyroid hormone over the course of treatment are shown in the appendix. As previously published, a one patient withdrew because of adverse events during the initial intravenous infusion of asfotase alfa and one patient died from sepsis at around age 8 months, after 7.5 months of therapy. No additional deaths or discontinuations occurred. All 11 patients had at least one treatment-emergent adverse event. The table summarises the treatment-emergent adverse events occurring in more than 25% of patients, regardless of association with asfotase alfa. The most common treatment-emergent adverse events were pyrexia, upper respiratory tract infection, craniosynostosis, pneumonia, constipation, otitis media, and vomiting. Most events were mild (605 [76%] of 794 events) or moderate (151 [19%] of 794 events) in severity; eight (73%) of 11 patients had severe treatment-emergent adverse events (38 events; appendix). Most events were considered by investigators to be unrelated to the study drug (664 [84%] of 794 events). Events assessed by investigators as possibly, probably, or definitely related to asfotase alfa were severe chronic hepatitis (n=1; this event occurred concurrently with use of montelukast), moderate immediate post-injection reaction (IAR, including abdominal pain, skin erythema, dizziness, headache, and chills; n=1), and severe craniosynostosis with severe conductive deafness (n=1).

Figure 4: Median length or height and weight Z scores over time in infants and young children with hypophosphatasia treated with asfotase alfa
Shaded area is the normal range (mean ±2 SD) for healthy age-matched and sex-matched peers. *p<0.05 for comparison with baseline.

<table>
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<th>Duration of treatment</th>
<th>Length or height Z score</th>
<th>Weight Z score</th>
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<td>Baseline</td>
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<td>Median (range)</td>
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<td>(n=11)</td>
<td>-2.72 (-9.2 to -0.7)</td>
<td>-3.84 (-9.4 to -0.5)</td>
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<td>Month 6</td>
<td>-3.62 (-8.2 to -1.8)</td>
<td>-4.35 (-6.4 to -1.5)</td>
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<tr>
<td>(n=10)</td>
<td>+0.18 (+0.4 to +1.1)</td>
<td>+0.32 (+0.6 to +1.0)</td>
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<td>Year 1</td>
<td>-2.85 (-8.2 to -3.2)</td>
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<td>(n=9)</td>
<td>+0.62 (+1.0 to +1.4)</td>
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<td>Year 2</td>
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<td>Year 3</td>
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<tr>
<td>(n=9)</td>
<td>-1.64 (-1.4 to -1.6)</td>
<td>+2.02 (+1.8 to +2.4)</td>
</tr>
<tr>
<td>Year 5</td>
<td>-2.71 (-9.0 to -0.3)</td>
<td>-1.22 (-5.0 to -0.2)</td>
</tr>
<tr>
<td>(n=9)</td>
<td>-1.46 (-1.2 to -1.6)</td>
<td>+1.00 (+1.0 to +1.4)</td>
</tr>
<tr>
<td>Year 6</td>
<td>-2.47 (-8.6 to -0.5)</td>
<td>-1.00 (-5.6 to -0.1)</td>
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<tr>
<td>(n=9)</td>
<td>-1.24 (-1.2 to -1.6)</td>
<td>-0.99 (-3.7 to +0.5)</td>
</tr>
<tr>
<td>Year 7</td>
<td>-3.10 (-8.7 to -0.6)</td>
<td>-2.71 (-1.2 to -1.6)</td>
</tr>
<tr>
<td>(n=7)</td>
<td>-1.62 (-2.7 to +0.5)</td>
<td>-2.40 (-3.1 to +1.0)</td>
</tr>
</tbody>
</table>
moderate in severity; none were life-threatening. The one patient who withdrew from the study had IARs of piloerection, pyrexia, and chills during the intravenous infusion. Blood complement testing was done in two patients with IARs; neither had a clinically significant result.

Seven (64%) of 11 patients had 78 ISRs; the majority (47 [60%] of 78 events) occurred in two patients. No severe or serious ISRs were reported. One patient had treatment-emergent adverse events 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 in that location for approximately 1 year (dosage at the time of the adverse event was 2 mg/kg three times per week). The calcifications were considered to be possibly drug related, treated by rotating the injection sites, and resolved by the end of the study. Three patients had treatment-emergent adverse events related to lipohypertrophy, all were mild or moderate in severity, occurring after at least 2 years of treatment, and ongoing at study end.

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

One patient developed mild treatment-emergent adverse events of ectopic calcifications in the conjunctiva approximately 6.5 years after starting asfotase alfa treatment. No action was taken. The ectopic calcifications were considered to be possibly related to lipohypertrophy, all were mild or moderate in severity, occurring after at least 2 years of treatment, and ongoing at study end.

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Figure 5: Motor and cognitive function over time in infants and young children with hypophosphatasia treated with asfotase alfa
(A) Scaled median (range) BSID-III gross motor, fine motor, and cognitive subscale scores. The BSID-III was applied for patients younger than 43 months. Assessments were done on the basis of the patient’s chronological age and functional abilities. Shaded area is the 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. (B) PDMS-2 locomotion subscale standard scores for each individual patient (months indicate age at study entry). (C) BOT-2 running speed and agility scaled scores for each individual patient (months indicate age at study entry).


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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 treatment-emergent adverse event at approximately year 3. The patient was treated with oral potassium citrate two times per day for 3 months. Subsequent renal ultrasounds indicated small calcium deposits at year 4.5, year 5, and year 5.5 that did not meet the criteria for nephrocalcinosis and that were gone from year 6 through to study end.

Serum concentrations of calcium and phosphate over the course of treatment are shown in the appendix.

Eight (80%) of ten evaluable patients tested positive for asfotase alfa antibodies (maximum titre 2048) over the course of treatment; five (50%) of ten patients tested positive for neutralising antibodies. No dosage adjustments were made on the basis of the presence of antibodies, and the antibodies had no apparent effect on pharmacodynamic outcomes, improvements in skeletal manifestations, or other outcome measures (data not shown).

Discussion

In this Article we report the long-term (up to 7 year) safety and efficacy of asfotase alfa treatment for paediatric patients with life-threatening hypophosphatasia. Although mortality historically has been high in patients with perinatal and infantile hypophosphatasia, most of the 11 individuals in our study who began therapy as infants or young children showed rapid and substantial improvements in skeletal mineralisation 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 (ie, PPi and PLP) that were achieved after 6 months of treatment18 persisted throughout the study, except for transient elevations in median PLP concentrations. Moreover, plasma concentrations of PPi following treatment with asfotase alfa were above or near the lower limit of normal, and did not decrease to subnormal concentrations. 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 hypophosphatasia skeletal disease, made using two validated scales (RGI-C20 and RSS 21), supported 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 score 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 paediatric hypophosphatasia than does the RSS score, at least +2, indicating substantial healing, were reached during 6 months of treatment and were sustained through year 7. With longer treatment, these still prepubertal children might have further skeletal healing.

Further, the skeletal improvements were associated with sustained improvements in respiratory status. Although none of nine patients required respiratory support from year 4 through to study end, we note that several participants required prolonged support as

<table>
<thead>
<tr>
<th>Patients treated with asfotase alfa (n=11)</th>
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<tbody>
<tr>
<td>Pyrexia 8 (73%)</td>
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<tr>
<td>Upper respiratory tract infection 8 (73%)</td>
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<tr>
<td>Craniosynostosis 7 (64%)</td>
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<tr>
<td>Pneumonia 7 (64%)</td>
</tr>
<tr>
<td>Constipation 6 (55%)</td>
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<tr>
<td>Otitis media 6 (55%)</td>
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<tr>
<td>Vomiting 6 (55%)</td>
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<td>Diarrhoea 4 (36%)</td>
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<td>Increased urine calcium:creatinine ratio 3 (27%)</td>
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<td>Sleep apnoea syndrome 3 (27%)</td>
</tr>
<tr>
<td>Tracheitis 3 (27%)</td>
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<tr>
<td>Wheezing 3 (27%)</td>
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Table: Treatment-emergent adverse events reported for more than 25% of patients with hypophosphatasia treated with asfotase alfa

Data are presented as number of patients (%).
babies or young children to achieve this outcome (figure 3). An expanded cohort of 39 paediatric patients with perinatal or infantile hypophosphatasia that included the patients enrolled in this study (n=11) and in another multicentre, 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 years). These 39 treated patients were compared with 48 untreated historical control patients of similar age with regard to hypophosphatasia characteristics. Among the 39 treated patients, 21 (54%) required ventilator support; 14 (36%) at baseline and an additional seven (18%) soon after initiation of therapy. 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). Among the 21 treated patients ever requiring respiratory support, 16 (76%) survived and 12 (57%) were weaned from respiratory support. Improved skeletal mineralisation in treated patients was associated with improved respiratory status, and RGI-C scores of at least +2 (substantial or near-complete or complete healing) were achieved by all patients who were weaned from respiratory support.13 The results of our study are also consistent with those of an open-label study by Kitaoka and colleagues in 2017,31 who reported improved skeletal mineralisation in 13 Japanese patients with hypophosphatasia (median age at baseline 91 days, range 0 days to 34 years) treated with asfotase alfa.

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 or height Z scores and year 1 in median weight Z scores. Median weight Z scores were normalised from year 3 to the end of the study, whereas median length or 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 (appendix), which probably lowered their length or 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 might have been artificially low and improved when the children were able to sit independently, emphasising 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 (eg, head control, rolling, sitting, and walking), fine motor (eg, manipulating blocks, holding a cup, and cutting with scissors), and cognitive (eg, discriminating and classifying objects) subscales. Most patients also showed increases in scaled scores, indicating catch up to healthy peers in acquisition of new motor and cognitive skills. Eight patients completed scores 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 (eg, shuttle running and hopping). Previously, we reported significant improvements for children aged 6–12 years at baseline (n=13) with severe hypophosphatasia in growth (p≤0.0088) and motor function (p≤0.01) over 5 years of asfotase alfa treatment during a phase 2, open-label study and its extension.32 Afsotase 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 publication in 2012.33 The most common treatment-emergent adverse events generally reflected typical signs, symptoms, or complications of hypophosphatasia, and infections that commonly occur in healthy young children. Ophthalmological examinations, renal ultrasound, and anti-asfotase alfa antibody testing revealed no additional substantial issues associated with this treatment. Seven patients had 13 craniosynostosis-related events. These events were not unexpected, given that craniosynostosis is a common complication of hypophosphatasia,34 and would not be expected to reverse with asfotase alfa treatment. In a natural history study of patients with severe perinatal and infantile hypophosphatasia, the reported incidence of craniosynostosis was 61%.35 Monitoring guidance for patients with hypophosphatasia receiving treatment with asfotase alfa was published in 2017 by Kishnani and colleagues.36 Briefly, recommendations for safety monitoring include ISRs and any hypersensitivity reactions and lipodystrophy, and events of special interest that in severely affected patients can include hypercalcaemia or hypocalcaemia, craniosynostosis, ectopic calcifications of the conjunctiva, and nephrocalcinosis. Injection-site lipoatrophy and atrophy can be prevented or minimised by rotating injection sites among the abdominal, delioid, and thigh areas.37 Although patients who are severely affected by hypophosphatasia might have hypercalcaemia,38 improvements in skeletal mineralisation can require an increase in calcium intake upon initiation of asfotase alfa treatment (ie, hungry bone syndrome). Therefore, serum calcium and parathyroid hormone must be monitored in such patients, and additional calcium should be provided as needed.

Our study had several limitations; understandably, it was uncontrolled, because life-threatening hypophosphatasia...
was present, and it involved only a small number of patients manifesting the most severe forms of this rare genetic metabolic disorder. However, the improved survival documented in this Article for perinatal or infantile hypophosphatasia 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.23 Furthermore, motor and cognitive function were assessed in our study using different instruments, sometimes sequentially, on the basis of the patient’s age, functional capability, and physical status. Lastly, age-equivalent or standard scores might not always capture functional improvements observed through increases in raw point scores.

To summarise, infants and young children with life-threatening perinatal or infantile hypophosphatasia treated with asfotase alfa before or at age 3 years showed substantial early improvements in skeletal mineralisation 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.

Contributors
MPW, DP, KPF, and SM helped design the study. MM, JT, JHS, NJS, and NB served as study investigators. MM, JT, JHS, NJS, and SM collected and assembled the data. SM analysed the data. All authors were involved in the data interpretation. MPW advanced the manuscript, coordinating the reviews and providing oversight on all revisions. WHM reviewed and selected all the radiographs for publication. All authors contributed to the review and revisions of the manuscript and approved the final version.

Declaration of interests
MPW was the principal clinical study investigator and received honoraria, travel support, and research grants from Alexion Pharmaceuticals. JHS was a clinical study investigator and received honoraria and travel support from Alexion Pharmaceuticals. SM and KPF are employees of and might own stock options in Alexion Pharmaceuticals. NJS was a clinical study investigator and received grant and research support from Alexion Pharmaceuticals. JT was a clinical study investigator and an employee of Prevea Health Clinic, which at the time of the study was owned by Hospital Sisters Health System St Vincent Hospital, which received grant and research support from Alexion Pharmaceuticals. DP was a consultant for Alexion Pharmaceuticals at the time of the study, and received funding and travel support from Alexion Pharmaceuticals for consulting and participating on advisory boards. MM was a clinical study investigator and received honoraria and travel support from Alexion Pharmaceuticals. WHM declares no competing interests.

Acknowledgments
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References