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

3

4 Michael P. Whyte, MD^{1,2}; Jill H. Simmons, MD³; Scott Moseley, MS, MS⁴; Kenji P.
5 Fujita, MD⁴; Nicholas Bishop, MD⁵; Nada J. Salman, MD⁶; John Taylor, DO⁷;
6 Dawn Phillips, PhD⁸; Mairead McGinn, MD⁹; William H. McAlister, MD¹⁰

7

8 ¹ Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for
9 Children, St. Louis, MO, USA

10 ² Division of Bone and Mineral Diseases, Department of Internal Medicine, Washington
11 University School of Medicine at Barnes-Jewish Hospital, St Louis, MO, USA
12 [mwhyte@shrinenet.org]

13 ³ Vanderbilt University Medical Center, Department of Pediatrics, Nashville, TN, USA
14 [jill.h.simmons@vanderbilt.edu]

15 ⁴ Alexion Pharmaceuticals, Inc., Boston, MA, USA [kenji.fujita@alexion.com;
16 scott.moseley@alexion.com]

17 ⁵ Sheffield Children's Hospital, Sheffield, UK [n.j.bishop@sheffield.ac.uk]

18 ⁶ Tawam Hospital, Al Ain, United Arab Emirates* [njsalman@hotmail.com]

19 ⁷ Prevea Health Clinic, HSHS St. Vincent Hospital, Green Bay, WI, USA
20 [john.taylor@prevea.com]

21 ⁸ University of North Carolina, Division of Physical Therapy, Chapel Hill, NC*
22 [dawnphillipspt@gmail.com]

1 ⁹ Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK

2 [mairead.mcginn@belfasttrust.hscni.net]

3 ¹⁰Mallinckrodt Institute of Radiology, Washington University School of Medicine, St.

4 Louis, MO, USA [mcalisterw@mir.wustl.edu]

5 * Affiliation at the time of the study.

6

7 **Corresponding Author:**

8 Michael P. Whyte, MD

9 Shriners Hospital for Children

10 4400 Clayton Avenue

11 St. Louis, MO, USA, 63110

12 Tel: 314-872-8305

13 Fax: 314-872-7844

14 Email: mwhyte@shrinenet.org

15

1 **Research in context**

2 ***Evidence before this study***

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

19

20 ***Added value of this study***

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

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

11

12 ***Implications of all the available evidence***

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

1 **ABSTRACT**

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

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

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

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

1 **Funding:** This study was sponsored by Alexion Pharmaceuticals, Inc.

2

3 **Keywords:** alkaline phosphatase; ambulation; enzyme replacement; inorganic

4 pyrophosphate; metabolic bone disease; osteomalacia; pyridoxal 5'-phosphate; rickets

5

1 [Word count limit: ~3500; current count: 5160]

2 INTRODUCTION

3 Hypophosphatasia (HPP) is the rare, inherited, metabolic bone disease caused by loss-
4 of-function mutation(s) of the *ALPL* gene that encodes the tissue-nonspecific isoenzyme
5 of alkaline phosphatase (TNSALP).^{1,2} Low TNSALP activity on cell surfaces results in
6 extracellular accumulation of its substrates, including inorganic pyrophosphate (PPi)
7 and pyridoxal 5'-phosphate (PLP).^{1,3-5} PPi potently inhibits mineralization by blocking
8 hydroxyapatite crystal formation.^{6,7} Thus, the superabundance of PPi in HPP often
9 leads to rickets during growth.^{6,7} TNSALP dephosphorylates PLP (the principal
10 circulating form of vitamin B6) to pyridoxal, which allows it to cross cell plasma
11 membranes and be rephosphorylated intracellularly to PLP. Thus, vitamin B6–
12 dependent seizures occur in some severely affected babies.^{4,7-9} Life-threatening
13 complications in the severe perinatal and infantile forms of HPP can include respiratory
14 failure from rachitic chest deformity and rib fractures, elevated intracranial pressure due
15 to craniosynostosis, and hypercalcemia leading to nephrocalcinosis and renal
16 compromise.^{7,10-12} Other potential complications in pediatric HPP include long bone
17 deformity and muscle weakness.^{7,10} Perinatal HPP historically has been considered
18 lethal, and infantile HPP has ~50% mortality during the first year of life.¹³⁻¹⁵
19
20 Asfotase alfa (Strensiq[®]; Alexion Pharmaceuticals, Inc., Boston, MA) is a human,
21 recombinant, TNSALP replacement therapy approved multinationally in 2015, typically
22 for pediatric-onset HPP.^{16,17} The safety and efficacy of asfotase alfa were first evaluated
23 during our Phase 2, open-label study in pediatric patients ≤3 years of age with life-

1 threatening perinatal or infantile HPP.¹⁸ This study enrolled 11 HPP patients (5 with
2 perinatal HPP and 6 with infantile HPP) ranging in age from 2 weeks to 3 years for the
3 6-month initial trial.¹⁸ Patients manifested complications of HPP before 6 months of age,
4 including skeletal abnormalities such as shortened or bowed limbs, rachitic chest
5 deformity, fractures, osteopenia, craniosynostosis, and/or other rachitic findings. All but
6 one patient had failure to thrive, most (82%) required respiratory support, and all had
7 gross motor delay. A single 2 mg/kg intravenous (IV) infusion of asfotase alfa preceded
8 subcutaneous (SC) injections starting at 1 mg/kg thrice weekly. Results from this study
9 published in 2012 showed outcomes after ≥ 12 months (range: 12–26 mo) of treatment
10 with asfotase alfa.¹⁸ One patient had consent withdrawn on Day 1 because of infusion-
11 associated reactions (IARs), and one patient died of pneumonia and sepsis after 7·5
12 months of treatment.^{18,19} The study met its primary efficacy measure of change in HPP
13 skeletal disease severity from Baseline to Month 6 based on assessment of skeletal
14 radiographs using the validated 7-point (-3=severe worsening; +3=complete/near
15 complete healing) Radiographic Global Impression of Change (RGI-C) scale (median
16 [min, max]: +2·0 [0, +2·3]; $p=0\cdot004$). Skeletal healing was accompanied by
17 improvements in secondary outcome measures of respiratory and motor function over 1
18 year of treatment. Asfotase alfa was generally well tolerated.¹⁸

19

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

23

1 **METHODS**

2 **Study design**

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

6

7 The study design, including patient inclusion and exclusion criteria, has been
8 published.¹⁸ The patient numbering scheme used in that publication is continued in our
9 current report. Briefly, this Phase 2, open-label study was conducted at 10 sites (six in
10 the United States, two in the United Kingdom, one in Canada, and one in the United
11 Arab Emirates). In the 6-month primary treatment period, safety and efficacy of asfotase
12 alfa were evaluated in infants and young children (≤ 3 years of age) with life-threatening
13 perinatal or infantile HPP (clinicaltrials.gov identifier: NCT00744042; EudraCT number:
14 2008-007406-11). A single 2 mg/kg IV infusion of asfotase alfa preceded 1 mg/kg SC
15 injections of asfotase alfa thrice weekly (total dosage: 3 mg/kg/wk). The dosage could
16 be increased to up to 3 mg/kg thrice weekly (total dosage: 9 mg/kg/wk) after 1 month for
17 lack of efficacy, defined as worsening of failure to thrive, deteriorating pulmonary
18 function, or no radiographic evidence of skeletal improvement. The extension phase
19 (NCT01205152; 2009-009369-32) continued the SC asfotase alfa dosage from the
20 primary treatment period; dosage adjustments were made at each visit for changes in
21 the patient's weight. Additional dosage adjustments (no limits on maximum dosage)
22 were permitted for lack of efficacy or for safety-related concerns. Patients continued to

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

3

4 **Efficacy outcome measures**

5 *Change in HPP skeletal disease:* HPP skeletal manifestations were evaluated using
6 sequential radiographs of the chest, wrists, and knees obtained at Baseline; Months 1,
7 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
8 paediatric radiologists independently rated changes compared with Baseline using the
9 RGI-C scale (-3=severe worsening, 0=no change, and +3=complete/near complete
10 healing), which has been validated in pediatric patients with HPP.²⁰ The mean of the
11 raters' RGI-C scores was calculated for each patient at each time point. A separate
12 rater independently evaluated the radiographs of the wrists and knees at each time
13 point using the 10-point Rickets Severity Scale (RSS; 0=absence of metaphyseal
14 cupping and fraying to 10=severe rickets; maximum of 4 points for wrists and 6 points
15 for knees), developed and validated to assess nutritional rickets in children (mean age:
16 4·5 years).²¹ All raters were blinded to all treatment time points (except Baseline
17 radiographs for RGI-C) and all other patient information.

18

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

22

1 *Growth:* Length/height, weight, and head circumference were recorded at study visits.
2 Z-scores for length/height and weight were determined using Centers for Disease
3 Control and Prevention growth charts for age- and sex-matched healthy infants and
4 children.²² Head circumference Z-scores were calculated using World Health
5 Organization (WHO) formulae.²³
6
7 *Motor and cognitive function:* Depending on the patient's age and functional abilities at
8 individual visits, each site's physical therapist, in consultation with the Medical Monitor,
9 determined the appropriate assessment or combination of assessments of motor and
10 cognitive development, which included the Bayley Scales of Infant and Toddler
11 Development, Third Edition (BSID-III); the Peabody Developmental Motor Scales,
12 Second Edition (PDMS-2); or the Bruininks-Oseretsky Test of Motor Proficiency,
13 Second Edition (BOT-2) (**Figure 1**). Patients ≤ 42 months of age, or older but with
14 severe developmental delays, were assessed using the Gross Motor, Fine Motor, and
15 Cognitive subscales of the BSID-III.²⁴ If patients demonstrated cognitive age-
16 equivalence of 42 months, such testing was discontinued. Patients 43–71 months of
17 age considered to have evaluable functional abilities were studied using the Locomotion
18 subtest of the PDMS-2, an assessment of gross motor skills.²⁵ Patients ≥ 72 months of
19 age with evaluable functional abilities completed the Running Speed and Agility and
20 Strength subtests of the BOT-2, an assessment of gross motor proficiency.²⁶ Licensed
21 physical therapists, or their local equivalents, conducted the assessments at Baseline
22 (BSID-III only), Months 3 (BSID-III only) and 6, Year 1, and every 6 months thereafter.

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

3

4 **Pharmacodynamic outcome measures**

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

19

20

21

1 **Safety and tolerability**

2 Adverse events (AEs), including injection site reactions (ISRs) and injection-associated
3 reactions (IARs), were continuously monitored. ISRs were defined as AEs localized to
4 the site of asfotase alfa administration, and IARs were defined as systemic signs,
5 symptoms, or findings (e.g., chills, cough, erythema) occurring within 3 hours after
6 asfotase alfa administration. The site investigator assessed the “possible,” “probable,”
7 or “definite” relationship of AEs to the study drug. Additional safety assessments
8 included physical examination findings, laboratory values (including calcium and
9 phosphate), and anti-asfotase alfa antibody testing results (the latter performed by
10 Cambridge Biomedical Inc., Boston, MA, USA, and PPD Laboratories, Richmond, VA,
11 USA). Patients were assessed for ectopic calcification by periodic funduscopy
12 examinations (changed to full ophthalmologic examinations by protocol amendment
13 after ~1.5 years) and periodic renal ultrasounds.

14

15 **Statistical analyses**

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

22

1 Median (min, max) values were calculated for the RGI-C and RSS scores; length/height,
2 weight, and head circumference raw values and Z-scores; and BSID-III scaled scores
3 over time. A 1-sample Wilcoxon signed-rank test using a 2-sided alpha of 0.05 was
4 used to test whether the median RGI-C score at each time point differed from 0 (i.e., no
5 change). The proportion of patients with RGI-C scores of +2 or +3 (RGI-C “responders”)
6 was calculated for each time point. Mean changes from Baseline in Z-scores for
7 length/height and weight were analyzed using a 1-sample *t*-test. Age-equivalent and
8 scaled (or standard) scores were calculated for each subscale/subtest of the BSID-III,
9 PDMS-2, and BOT-2. Scaled or standard scores for each were then compared with the
10 normative mean (SD) values for healthy age-matched peers, which were 10 (3) for the
11 BSID-III scaled score,²⁴ 10 (3) for the PDMS-2 standard score,²⁵ and 15 (5) for the BOT-
12 2 Running Speed and Agility subtest scaled score.²⁶ These functional measures were
13 exploratory and therefore not analyzed statistically.

14

15 Pharmacodynamic and safety outcome measures are summarized descriptively.

16

17 **Role of the funding source**

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

20

1 RESULTS

2 Patients

3 Eleven patients were enrolled and received at least one dose of asfotase alfa (median
4 age at enrollment: 30 weeks [min: 3; max: 158 [~3 y]; sex: 64% female). All ten patients
5 who completed the 6-month primary treatment period entered the extension phase;
6 nine received asfotase alfa for ≥ 6 years and completed the study, with four of the nine
7 treated for >7 years. Patients were enrolled and received treatment between October
8 2008 and August 2016. The median duration of treatment for the 11 enrolled patients
9 was 6.6 years (range: 0.003–7.5). Patient demographics, Baseline characteristics, and
10 efficacy and safety outcomes for nine patients through ≥ 1 year of treatment and detailed
11 case reports of all 11 enrolled patients were published.¹⁸ Updated narratives for all
12 patients who completed the study are provided in the on-line **Supplementary**
13 **Appendix** to this article.

14
15 Four patients had major protocol deviations considered to potentially influence
16 treatment effect and were therefore excluded from the PP population. Two did not meet
17 the ALP eligibility criterion (ALP activity ≤ 3 SD below the mean for age). One did not
18 meet the failure to thrive eligibility criterion (too young developmentally for assessment)
19 and did not receive a weight-adjusted dose of asfotase alfa (received 8 mg SC
20 injections thrice weekly) from approximately Week 9 until his death at Week 23, with his
21 last recorded weight being 5.7 kg. One patient had dosage increases for failure to thrive
22 on Day 22 (from 1 mg/kg thrice weekly to 1.5 mg/kg [one dose only]) and on Day 24 (to

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

3
4 Because primary efficacy results (RGI-C scores at Month 6) were similar between the
5 FA and PP populations (data not shown), we present results after 6–7 years of asfotase
6 alfa treatment for the FA population only.

7

8 **Efficacy Outcome Measures**

9 *Changes in skeletal manifestations of HPP*

10 Median RGI-C scores documented improvements in HPP skeletal disease as early as
11 Month 3 that were typically sustained over 7 years of treatment, with statistically
12 significant ($p < 0.05$) gains at most visits (**Figure 2A**). The proportion of evaluable
13 patients with RGI-C scores $\geq +2$ (responders) was 89% (8/9) at Year 1 and 100% (7/7)
14 at Year 7. The highest possible RGI-C score of +3 was achieved by four patients, with
15 three first achieving a +3 by Year 1 and one first achieving a +3 at Year 2; all four
16 maintained scores $\geq +2$ at all time points thereafter.

17

18 Median RSS scores confirmed that the improvements documented as early as the sixth
19 month¹⁸ were sustained over 7 years of treatment, with significant ($p < 0.05$) decreases
20 indicating improvement from Baseline at most visits (**Figure 2B**).

21

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

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

6 *Respiratory support*

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

17 *Growth*

18 Median (min, max) length/height was 56·5 (39·0, 83·0) cm at Baseline (n=11) and 112·5
19 (88·1, 123·0) cm at Year 7 (n=7). The median length/height Z-score was higher than at
20 Baseline from Month 6 through Year 7, although the value remained >2 SD below the
21 mean for healthy age- and sex-matched peers at all time points (**Figure 5**). Overall, four
22 of nine patients had Z-scores within the normal range at Last Assessment. The mean

1 increase from Baseline in length/height Z-score was statistically significant at Year 3
2 ($p=0.0385$) and Year 4-5 ($p=0.0346$), but not at other time points.

3
4 Median (min, max) weight was 4.1 (2.1, 9.2) kg at Baseline ($n=11$) and 19.8 (15.1,
5 31.4) kg at Year 7 ($n=7$). Median weight Z-scores increased to within 2 SD of the mean
6 for healthy age- and sex-matched peers at most time points from Years 3 through 7
7 (**Figure 5**). The mean increase from Baseline in weight Z-score was statistically
8 significant at Year 3 ($p=0.0096$) and Year 4-5 ($p=0.0074$), but not at other time points.

9
10 Median (min, max) head circumference was 41.5 (33.0, 47.6) cm at Baseline ($n=11$)
11 and 50.5 (44.5, 51.3) cm at Year 7 ($n=7$). Head circumference Z-scores remained
12 stable, with a median (min, max) value of -1.01 ($-4.0, 0.8$) at Baseline ($n=11$) and
13 -1.34 ($-4.1, 1.0$) at Last Assessment ($n=10$; WHO criteria allow for calculation of head
14 circumference Z-scores for patients aged ≤ 5 years).

15
16 *Motor and cognitive function*

17 *BSID-III*: At Baseline or first assessment, 82% (9/11) of patients had BSID-III Gross
18 Motor scaled scores of 1 (3 SDs below the normative mean). Nine patients had serial
19 BSID-III assessments (two patients had one assessment each because of
20 discontinuation and death) (**Table S1**). All nine showed improvements in age-equivalent
21 scores on the Gross Motor, Fine Motor, and Cognitive subscales (**Table S2**). Median
22 (min, max) scaled Gross Motor scores improved from 1.0 (1, 8) at Baseline to 6.0 (2, 8)
23 at Year 3 (normative mean [SD]: 10 [3]; **Figure 6A**), indicating motor skill improvement

1 and less developmental delay. Median scores on the Fine Motor and Cognitive
2 subscales were low at Baseline but normalized at Years 2 and 3 (**Figure 6A**).
3
4 *PDMS-2*: Eight patients advanced to complete serial PDMS-2 assessments (**Table S1**).
5 All demonstrated continued motor skill acquisition on the PDMS-2 Locomotion subtest
6 (i.e., increased age-equivalent scores [**Figure S1**]). Among them, seven had standard
7 scores >1 SD below the normal reference range (score: <7) when they first completed
8 the assessment; five achieved scores within 1 SD of normal (**Figure 6B**).
9
10 *BOT-2*: Eight patients transitioned to the BOT-2 and completed at least one BOT-2
11 assessment (**Table S1**); all had received asfotase alfa for ≥ 5 years when first tested.
12 Seven had initial scaled BOT-2 Running Speed and Agility subtest scores >1 SD below
13 normal (scaled score: <10) (**Figure 6C**). Three achieved normal scaled scores (≥ 10) by
14 study end. All six who completed serial BOT-2 assessments had increased age-
15 equivalent BOT-2 scores during treatment (**Table S3**).
16

17 **Pharmacodynamic Outcome Measures**

18 Baseline serum ALP activity was low (median [min, max]: 21 [9, 46] U/L) in the nine
19 evaluable patients, increased by approximately 100-fold by Day 2 after the single IV
20 infusion of asfotase alfa (2990 [449, 8007] U/L; n=9), and remained elevated thereafter
21 with SC dosing (e.g., 5304 [1812, 10085] U/L at Year 1 [n=8]¹⁸ and 4143 [2267, 6792]
22 U/L at Year 7 [n=7]).
23

1 Baseline plasma concentrations of PPi were elevated in four of eight evaluable patients
2 (median [min, max]: 5.2 [2.9, 10.5] μM ; normal range for patients aged 0–12 years:
3 1.33–5.71 μM) (**Figure S2A**). Median PPi levels decreased for all nine patients tested
4 at Month 3 of treatment (2.6 [1.0, 4.4] μM), and remained decreased relative to
5 Baseline at other study visits including Year 7 (4.6 [2.1, 10.2] μM ; n=7), with the
6 exception of isolated fluctuations for individual patients.

7
8 Baseline plasma concentrations of PLP were elevated in all nine evaluable patients
9 (median [min, max]: 421.0 [100.0, 880.0] ng/mL; normal range for patients aged 0–5 y:
10 11.8–68.4 ng/mL) (**Figure S2B**). Median PLP levels stayed within the normal range
11 from Month 6 (47.6 [16.4, 1510.0] ng/mL; n=10) to Year 7 (38.8 [19.1, 161.0] ng/mL;
12 n=7; normal range for patients aged 5–18 y: 5.7–61.2 ng/mL). Only one patient's PLP
13 value failed to correct at any time during the study (range: 81.3–960 ng/mL); this patient
14 was not receiving vitamin B6 supplementation. Overall, data appeared similar when
15 patients receiving vitamin B6 supplementation were excluded from the analysis (not
16 shown).

17
18 Serum concentrations of PTH over the course of treatment are shown in the
19 Supplementary Appendix (**Figure S3**).

21 **Safety**

22 As previously published,¹⁸ one patient withdrew because of AEs during the initial IV
23 infusion of asfotase alfa and one patient died from sepsis at ~8 months of age after 7.5

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

18
19 Four patients had a total 10 AEs considered by the investigators as possibly reflecting
20 hypersensitivity that were therefore designated IARs. Most (8/10) occurred on Day 1 in
21 conjunction with the initial IV infusion. The only IAR reported for more than one patient
22 was pyrexia (n=3). All IARs were mild or moderate in severity; none were life-
23 threatening. The one patient who withdrew from the study experienced IARs of

1 piloerection, pyrexia, and chills during the IV infusion. Blood complement testing was
2 performed in two patients with IARs; neither had a clinically significant result.
3
4 Seven patients (64%) experienced 78 ISRs; the majority (60% [47/78]) occurred in two
5 patients. No severe or serious ISRs were reported. One patient had TEAEs of injection
6 site calcifications observed radiographically in the soft tissue lateral to the left and right
7 hip joints after receiving asfotase alfa injections deeply and repeatedly there for
8 approximately 1 year (dosage at the time of the AE: 2 mg/kg thrice weekly). The
9 calcifications were considered possibly drug-related, treated by rotating the injection
10 sites, and resolved by study end. Three patients had TEAEs of lipohypertrophy, all mild
11 or moderate in severity, occurring after ≥ 2 years of treatment, and ongoing at study end.
12
13 Two patients had TEAEs of seizures during treatment; one had pyridoxine-dependent
14 seizures before and during the study. Six patients had fractures during the study; all but
15 one had a prestudy history of fractures. Seven patients had 13 craniosynostosis-related
16 events that were all moderate or severe and, in all but one patient, considered unrelated
17 to asfotase alfa treatment. Four patients underwent surgery for craniosynostosis.
18
19 One patient developed mild TEAEs of ectopic calcifications in the conjunctiva
20 approximately 6.5 years after starting treatment; no action was taken. The findings were
21 considered to be possibly related to asfotase alfa treatment and were ongoing at study
22 end. Two patients had TEAEs of nephrocalcinosis, observed when the asfotase alfa
23 dosage was 2 mg/kg thrice weekly. The first patient had a history of bilateral

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

9
10 Serum concentrations of calcium and phosphate over the course of treatment are
11 shown in the Supplementary Appendix (**Figure S3**).

12
13 Eight of 10 evaluable patients (80%) tested positive for anti-asfotase alfa antibodies
14 (maximum titer: 2048) over the course of treatment; 5 tested positive for neutralizing
15 antibodies. No dosage adjustments were made based on the presence of antibodies,
16 and the antibodies had no apparent effect on pharmacodynamic outcomes,
17 improvements in skeletal manifestations, or other outcome measures.

18

19 **DISCUSSION**

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

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

4
5 Pharmacodynamic results showed that decreases in the plasma concentrations of
6 TNSALP substrates (i.e., PPI and PLP) achieved by 6 months of treatment¹⁸ persisted
7 throughout the study, except for transient elevations in median PLP concentrations.
8 Moreover, plasma concentrations of PPI with treatment with asfotase alfa were above or
9 near the lower limit of normal and did not decrease to subnormal levels. Low PPI has
10 been associated with increased risk of vascular and other forms of ectopic calcification
11 in animal models and certain patient populations.²⁸⁻³⁰

12
13 Radiographic assessments of HPP skeletal disease, made using two validated scales
14 (RGI-C²⁰ and RSS²¹), confirmed improvement in all evaluable patients as early as
15 Month 6 of treatment.¹⁸ These patients had severe rickets at Baseline, with a median
16 RSS of 8·3. After 4 years of treatment, this was reduced to a median RSS score of 0·5,
17 which represents near absence of metaphyseal cupping or fraying. On the RGI-C scale,
18 which provides a broader assessment of the skeletal features of pediatric HPP, scores
19 of at least +2, indicating substantial healing, were reached during 6 months of treatment
20 and were sustained through Year 7. It may be that with longer treatment, these still
21 prepubertal children will experience further skeletal healing.

22

1 Further, the skeletal improvements were associated with sustained improvements in
2 respiratory status. As shown in **Figure 4**, although none of the nine patients required
3 respiratory support from Year 4 through study end, it is important to appreciate that
4 several as babies or young children required prolonged support to achieve this
5 outcome. An expanded cohort of 39 pediatric patients with perinatal/infantile HPP that
6 included those enrolled in the current study (n=11) or in another multicenter,
7 multinational, open-label study (n=28; age \leq 5 years) has been assessed for respiratory
8 function and survival during treatment with asfotase alfa (median duration: 2.7 y).¹⁵
9 These 39 treated patients were compared with 48 untreated historical control patients of
10 similar age and HPP characteristics. Among the 39 treated patients, 21 (54%) required
11 ventilator support: 14 (36%) at Baseline and an additional 7 (18%) soon after initiation of
12 therapy.¹⁵ Among the 48 historical controls, 20 (42%) required some form of respiratory
13 support. Kaplan-Meier estimated survival at 5 years was significantly better for the
14 treated patients (82%) than the historical controls (27%; $p < 0.0001$).¹⁵ Among the 21
15 treated patients ever requiring respiratory support, 16 (76%) survived and 12 (57%)
16 were weaned from respiratory support. Improved skeletal mineralization in treated
17 patients was associated with improved respiratory status, with RGI-C scores $\geq +2$
18 (substantial or near complete/complete healing) achieved by all who were weaned from
19 respiratory support.¹⁵ The results of our study are also consistent with those of an open-
20 label, study conducted by Kitaoka et al in 2017, who reported improved survival,
21 skeletal mineralization, and respiratory status in 13 Japanese patients with HPP
22 (median [min, max] age at Baseline: 91 d [0 d, 34 y]) treated with asfotase alfa.³¹

23

1 Long-term evaluation of the current cohort of nine treated children provided evidence of
2 catch-up growth in some patients. Improvements were observed as early as Month 6 in
3 median length/height Z-scores and Year 1 in median weight Z-scores. Median weight Z-
4 scores normalized from Year 3 through study end, whereas median length/height Z-
5 scores generally improved but remained below normal throughout the study.

6 Approximately half of the patients had craniosynostosis requiring surgery, and two
7 patients were found to have scoliosis (see **Supplemental Appendix**), which may have
8 lowered their length/height Z-scores.

9

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

1 advanced to the BOT-2 Running Speed and Agility subtest, which in itself reflects the
2 development of skills typical of school age children (e.g., shuttle run, hopping).
3 Previously, we reported significant improvements for children aged 6–12 years at
4 baseline (n=13) with severe HPP in growth ($p \leq 0.0088$) and motor function ($p \leq 0.01$) over
5 5 years of asfotase alfa treatment during a Phase 2, open-label study and its
6 extension.³²

7
8 Asfotase alfa continued to be generally well tolerated in this study. No deaths or safety-
9 related discontinuations of therapy occurred after the one death and one study
10 withdrawal discussed in our 2012 publication.¹⁸ The most common TEAEs generally
11 reflected typical signs, symptoms, or complications of HPP, as well as infections that
12 commonly occur in healthy young children. Ophthalmologic examinations, renal
13 ultrasound, and anti-asfotase alfa antibody testing revealed no additional significant
14 issues associated with this treatment. Seven patients had 13 craniosynostosis-related
15 events. This was not unexpected, as craniosynostosis is a common complication of
16 HPP¹¹ and would not be expected to reverse with asfotase alfa treatment. In a natural
17 history study of patients with severe perinatal and infantile HPP, the reported incidence
18 of craniosynostosis was 61%.³³

19
20 Monitoring guidance for patients with HPP receiving treatment with asfotase alfa was
21 published in 2017 by Kishnani et al.³⁴ In brief, recommendations for safety monitoring
22 include ISRs and any hypersensitivity reactions and lipodystrophy, as well as events of
23 special interest that in severely affected patients can include

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

9
10 Our study had several limitations. Understandably, it was uncontrolled as life-
11 threatening HPP was present, and it involved a small number of patients manifesting
12 the most severe forms of this rare inborn error of metabolism. However, the improved
13 survival documented here for perinatal/infantile HPP treated with asfotase alfa was
14 consistent with that found in a subsequent investigation of a larger cohort of similarly
15 treated patients that included a matched historical control group.¹⁵ Furthermore, motor
16 and cognitive function were assessed in this current study using different instruments,
17 sometimes sequentially, based on the patient's age, functional capability, and physical
18 status. Lastly, age-equivalent or standard scores may not always capture functional
19 improvements observed through increases in raw point scores.

20 21 **CONCLUSION**

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

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

4

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11

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13

14 **Declaration of interests**

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

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

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

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

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

3 **John Taylor** was a clinical study investigator and an employee of Prevea Health Clinic,
4 which at the time of the study, was owned by HSHS St. Vincent Hospital, which
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6 **Dawn Phillips** was a consultant for Alexion Pharmaceuticals, Inc., at the time of the
7 study and had received funding and travel support from Alexion Pharmaceuticals, Inc.,
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9 **Mairead McGinn** was a clinical study investigator and received honoraria and travel
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11 **William H McAlister** was a clinical study investigator and has not received any
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17 integrity of the data and the accuracy of the data analysis.

18

19 **Author contributions**

20 Study design: Michael P. Whyte, Scott Moseley

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

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

- 1 Collection and assembly of data: Mairead McGinn, John Taylor, Jill H. Simmons, Scott
- 2 Moseley
- 3 Data analysis: Scott Moseley
- 4 Data interpretation: All authors
- 5 Manuscript preparation: Michael P. Whyte
- 6 Manuscript review and revisions: All authors
- 7 Final approval of manuscript: All authors
- 8

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7

1 **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

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

7 -----
8 BSID-III=Bayley Scales of Infant and Toddler Development, Third Edition; PDMS-
9 2=Peabody Developmental Motor Scales, Second Edition; BOT-2=Bruininks-Oseretsky
10 Test of Motor Proficiency, Second Edition.

11 ^aValidated for impaired and healthy infants and toddlers aged 1–42 mo.²⁴

12 ^bValidated for ages ≤ 60 mo.²⁵

13 ^cValidated for ages 48–252 mo.²⁶

14

15 **Figure 2. Median RGI-C scores (A) and RSS scores (B) over time in infants and**
16 **young children with HPP treated with asfotase alfa.** Patients with RGI-C scores of
17 +2 (substantial healing of HPP rickets) or higher (complete healing) were classified as
18 RGI-C “responders.”¹⁸ *p<0·05 for change from Baseline based on Wilcoxon signed-
19 rank test. Significant improvement in RGI-C scores was demonstrated by Month 3 of
20 treatment, reaching a median score of +2·0 by Month 6¹⁸ that was sustained ≥+2·0
21 thereafter. Similarly, significant improvements in RSS scores observed as early as
22 Month 6¹⁸ were sustained at most visits over 7 years of treatment.

23 -----

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

3
4 **Figure 3. Representative radiographic changes spanning Baseline to Year 6.5 of**
5 **asfotase alfa treatment are illustrated for (A) the left wrist of patient #1 and (B) the**
6 **right knee of patient #2.** For both patients at Baseline, there are markedly widened
7 physes with indistinct provisional zones of calcification, and metaphyseal flaring
8 (*arrows*), as well as generalized osteopenia consistent with severe rickets. Substantial
9 improvements are apparent at Month 6 of asfotase alfa treatment and sustained at 6.5
10 years of therapy.

11
12 **Figure 4. Respiratory support spanning the entire study for infants and young**
13 **children with HPP treated with asfotase alfa.** Although nearly all patients required
14 respiratory support (sometimes prolonged) in the beginning,¹⁸ no support was required
15 by the nine patients beginning mid-Year 4 of treatment¹⁵ and extending to study
16 completion.

17 -----
18 CPAP=continuous positive airway pressure; BiPAP=bilevel or biphasic positive airway
19 pressure; HPP=hypophosphatasia.

20 ^a Noninvasive respiratory support included CPAP, BiPAP, and supplemental oxygen.

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

23

1 **Figure 5. Median length/height and weight Z-scores over time in infants and**
2 **young children with HPP treated with asfotase alfa.** Green shaded area reflects the
3 normal range (mean \pm 2 SD) for healthy age- and sex-matched peers. * $p < 0.05$ for
4 change from Baseline based on one-sample *t*-test. After Year 3 of treatment, median
5 weight normalized and remained normal through study end despite past failure to thrive
6 for many of the patients. Median length/height also improved but did not reach the
7 normal range.

8 -----
9 HPP=hypophosphatasia.

10
11 **Figure 6. Scaled BSID-III Gross Motor, Fine Motor, and Cognitive subscale scores**
12 **(A), PDMS-2 Locomotion subscale standard scores (B), and BOT-2 Running**
13 **Speed and Agility scaled scores (C) over time in infants and young children with**
14 **HPP treated with asfotase alfa.** The BSID-III was applied for patients aged <43
15 months. Assessments were performed based on the patient's chronologic age and
16 functional abilities. Green shaded area reflects mean (SD) scaled score for healthy age-
17 matched peers. Stability in scaled scores over time indicates continued acquisition or
18 improvement in quality of motor skills. **(A)** The BSID-III showed improvement in Gross
19 Motor scores by Year 2 of treatment, although scores did not normalize by Last
20 Assessment. BSID-III Fine Motor and Cognitive scores improved by Month 3 and
21 normalized at Year 2. **(B)** Of eight patients completing serial PDMS-2 assessments,
22 seven had standard scores below the normal range at first assessment and 5 achieved
23 normal scores at ~Year 4.5–5.5 of treatment. **(C)** Of eight patients transitioned to the

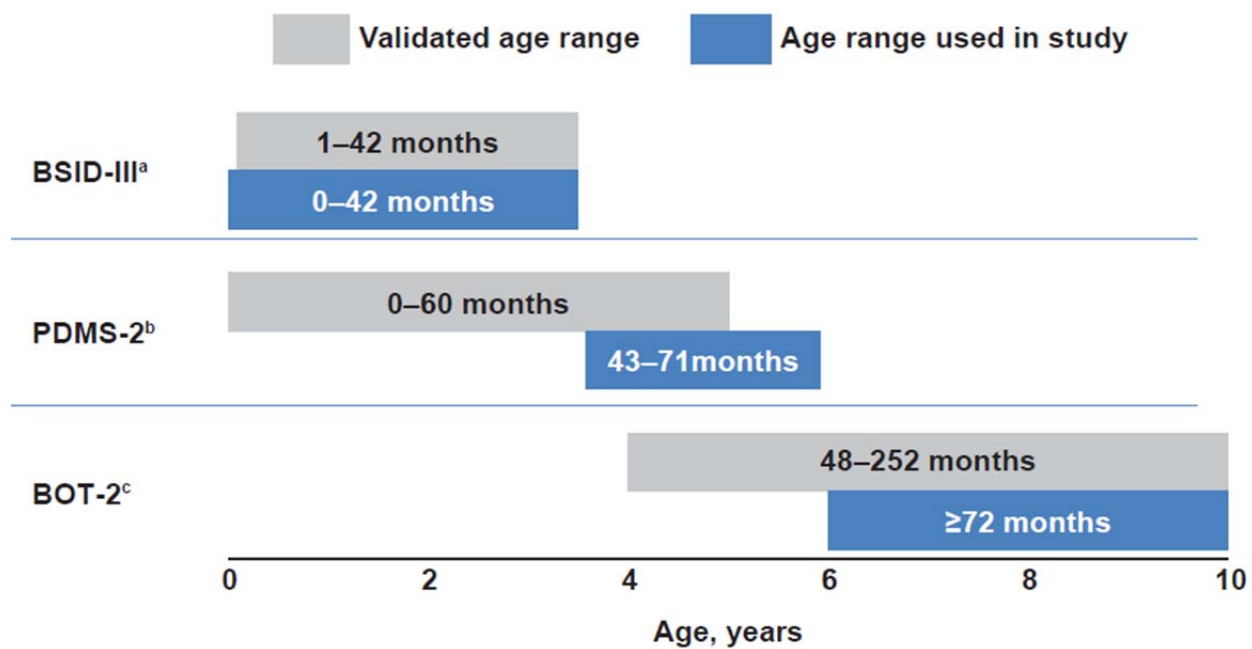
1 BOT-2, seven had Running Speed and Agility scores below the normal range at first
2 assessment and three had normal scaled scores at ~Years 6–8.

3 -----

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

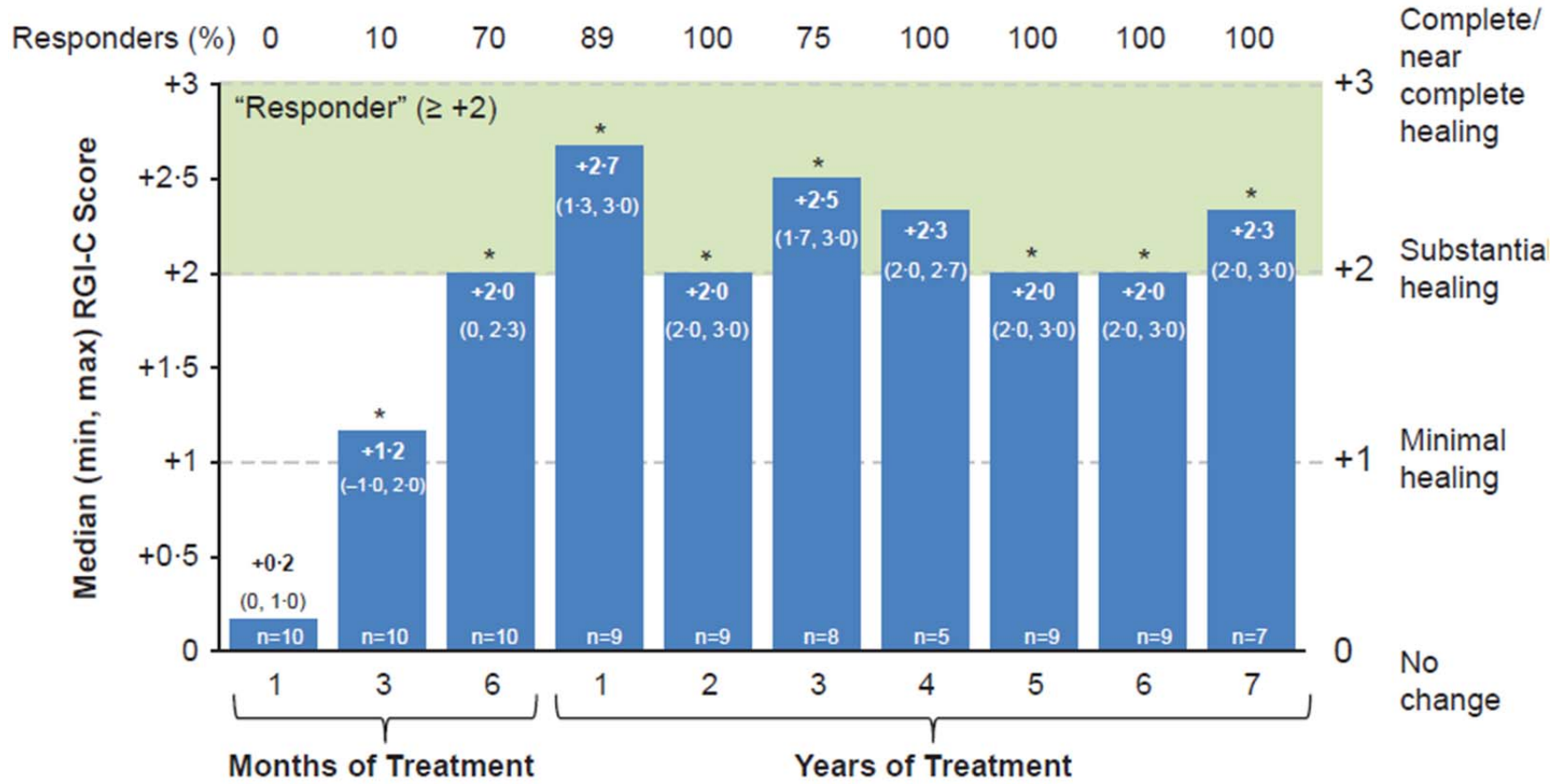
7

1 **Figure 1**



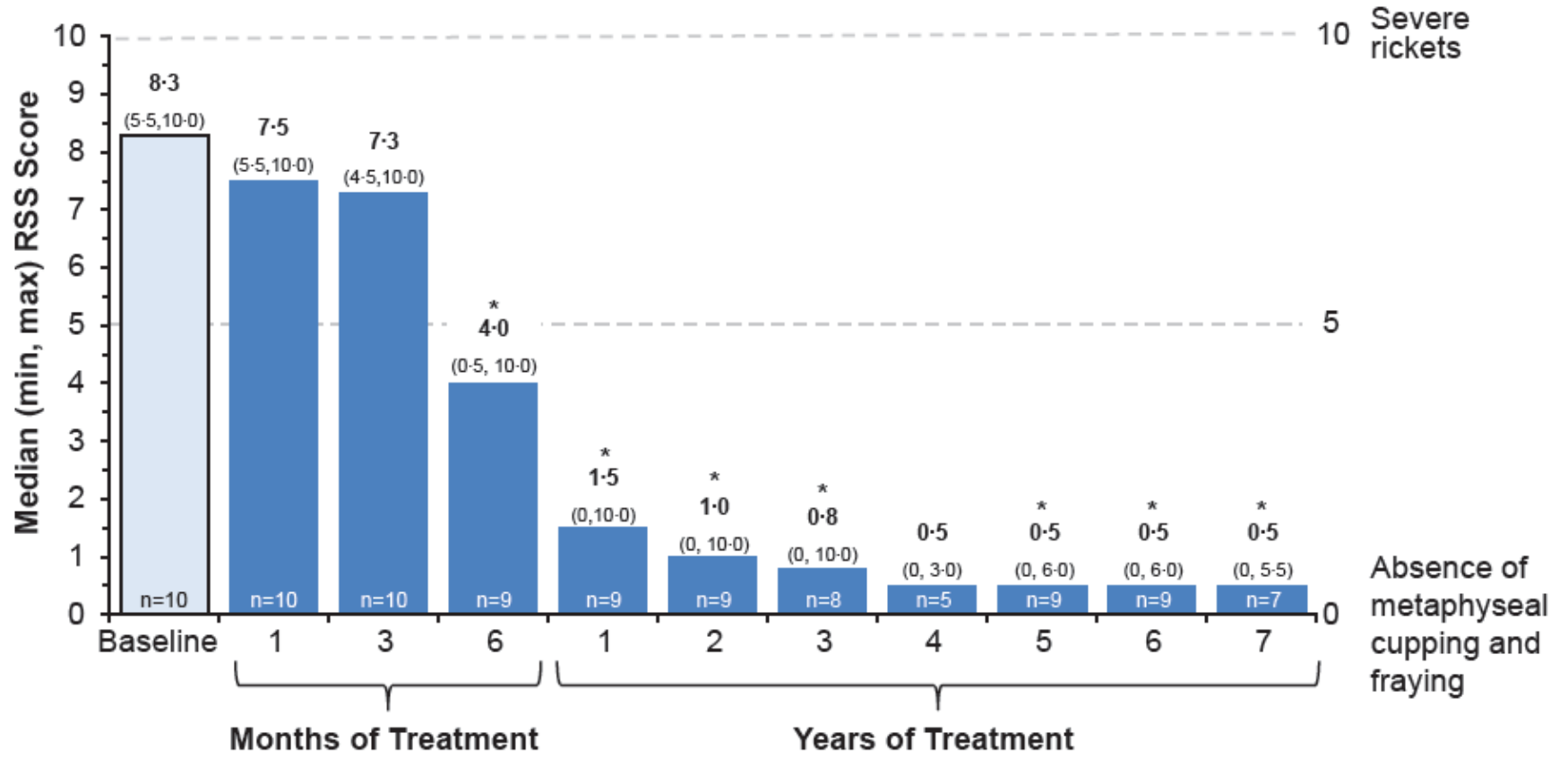
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1 **Figure 2A**



2

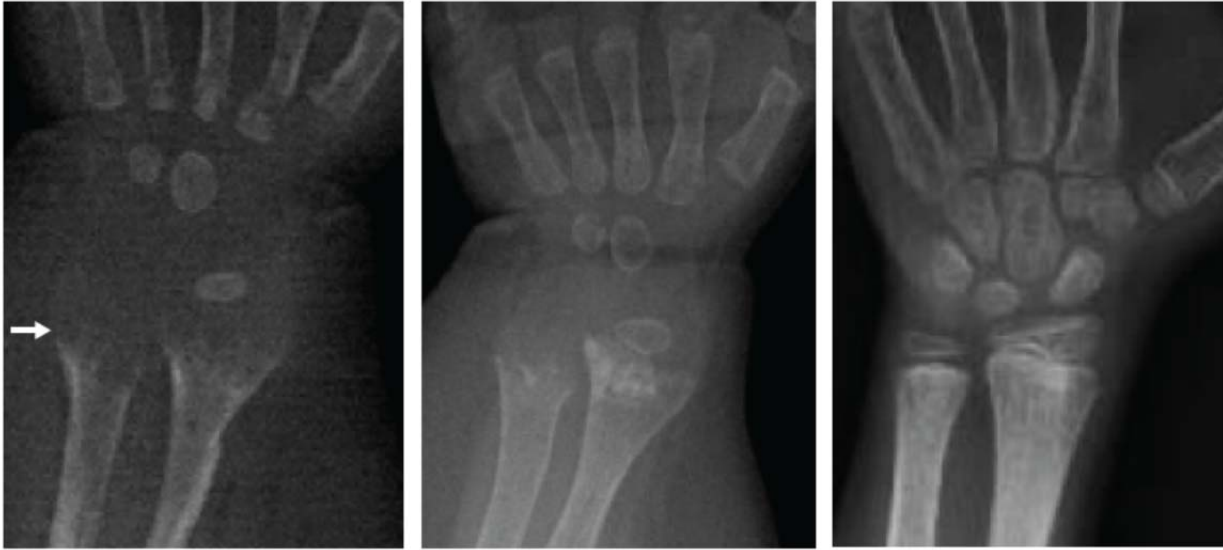
1 **Figure 2B**



2

1 **Figure 3A**

Patient #1



2 **Baseline**

Month 6

Year 6.5

3 **Figure 3B**

Patient #2

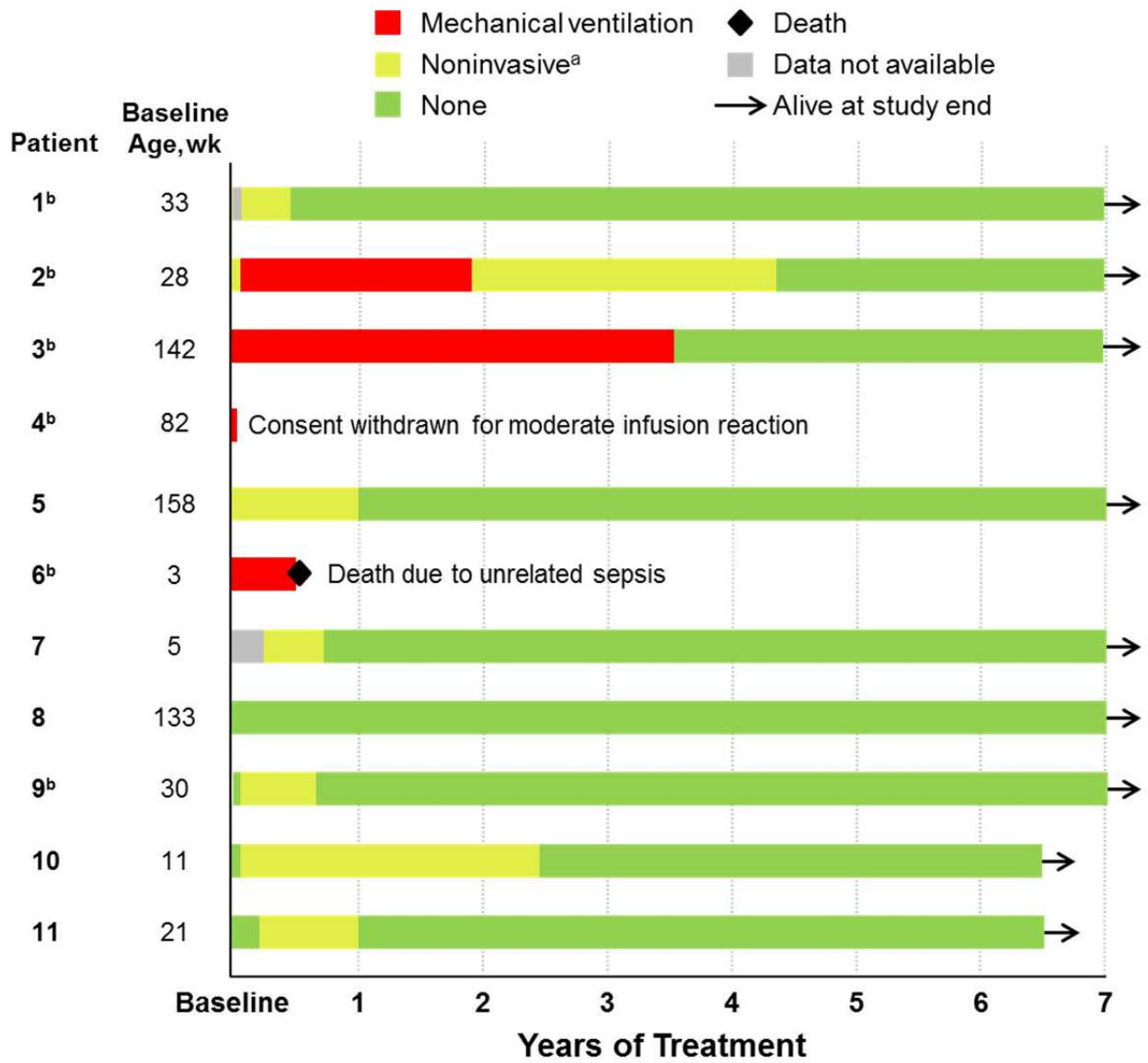


4 **Baseline**

Month 6

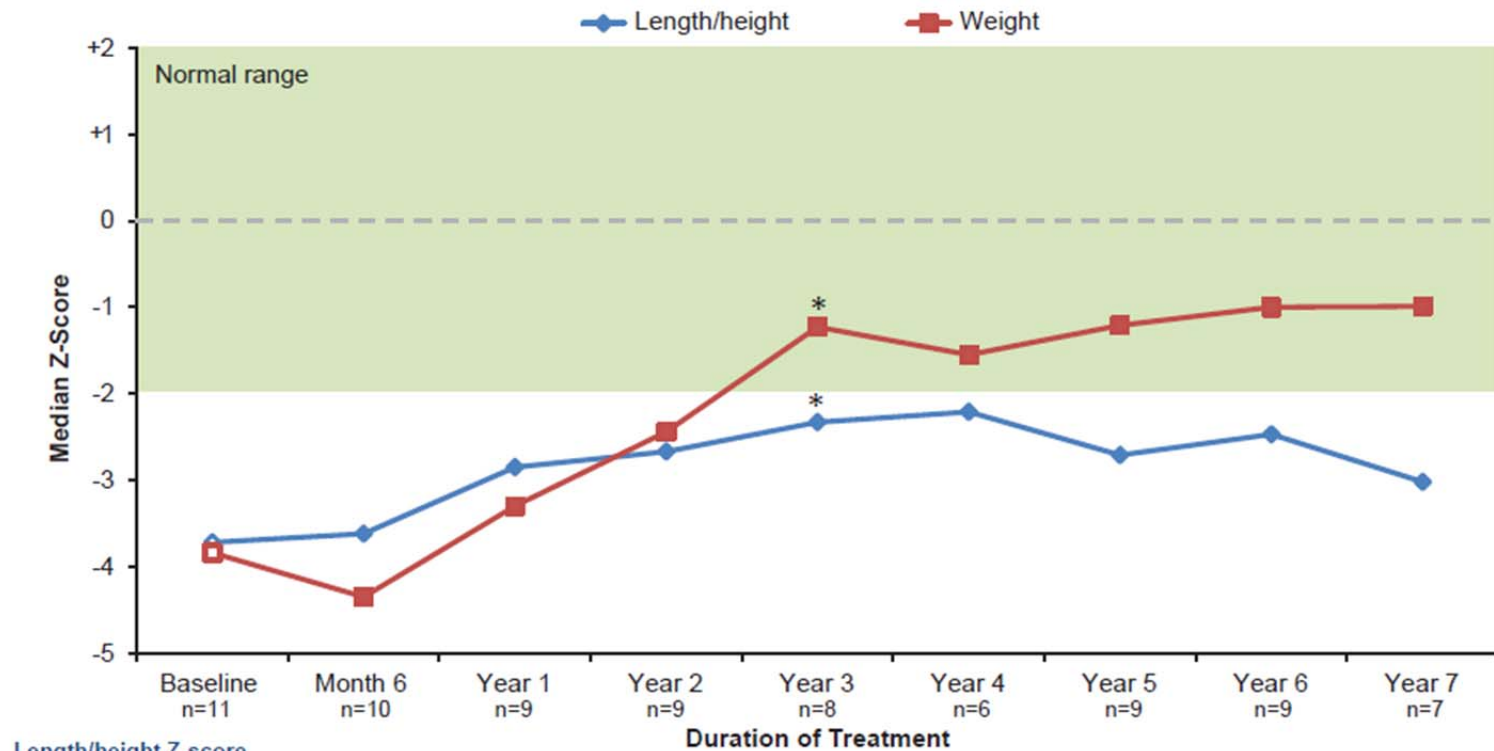
Year 6.5

1 **Figure 4**



2

1 **Figure 5**



Length/height Z-score

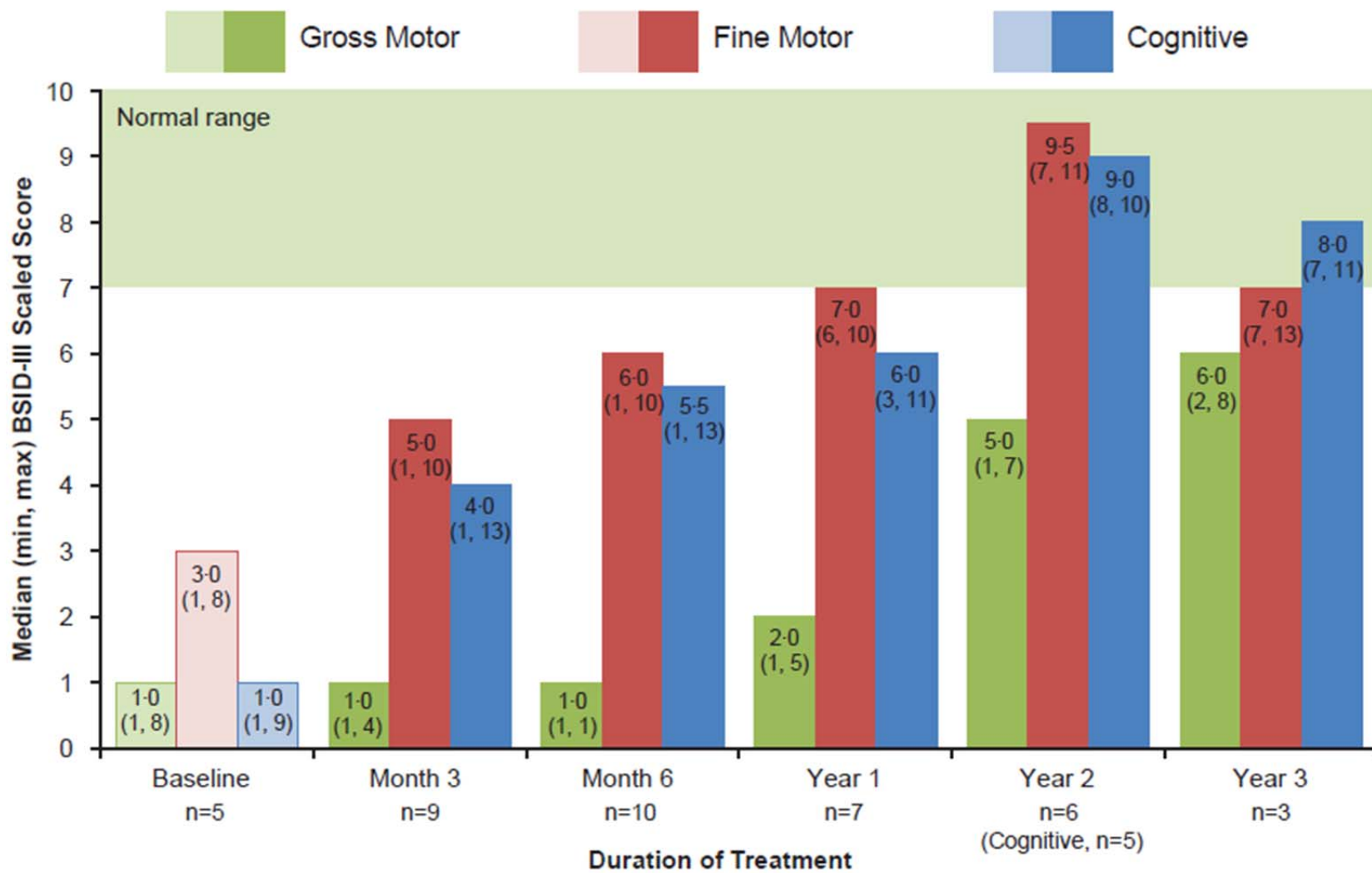
Median	-3.72	-3.62	-2.85	-2.67	-2.33	-2.21	-2.71	-2.47	-3.02
(min, max)	(-9.2, -0.7)	(-8.2, -1.8)	(-9.2, -1.2)	(-8.4, -1.0)	(-8.6, -0.4)	(-5.0, +0.3)	(-9.0, +0.1)	(-8.6, -0.5)	(-8.7, -0.6)
Change from Baseline, Mean (95% CI)		+0.18 (-0.60, +0.97)	+0.62 (-0.27, +1.52)	+1.00 (-0.43, +2.42)	+1.69 (+0.12, +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	-3.84	-4.35	-3.30	-2.44	-1.23	-1.55	-1.21	-1.00	-0.99
(min, max)	(-5.4, -0.5)	(-6.4, -1.5)	(-6.3, -1.7)	(-4.8, -0.9)	(-5.1, 0.4)	(-3.6, -0.8)	(-5.0, +0.2)	(-5.6, -0.1)	(-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)

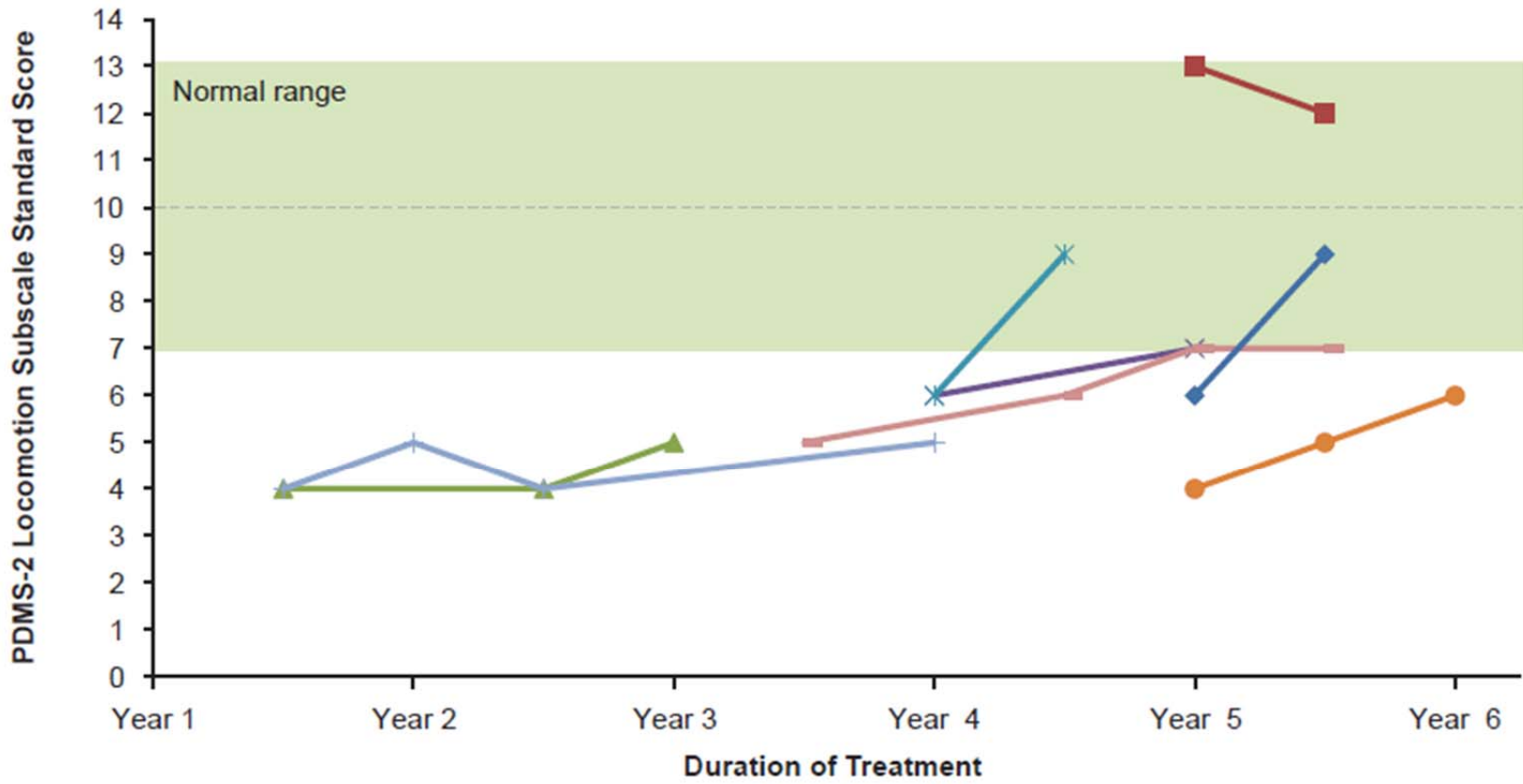
2

1 Figure 6A



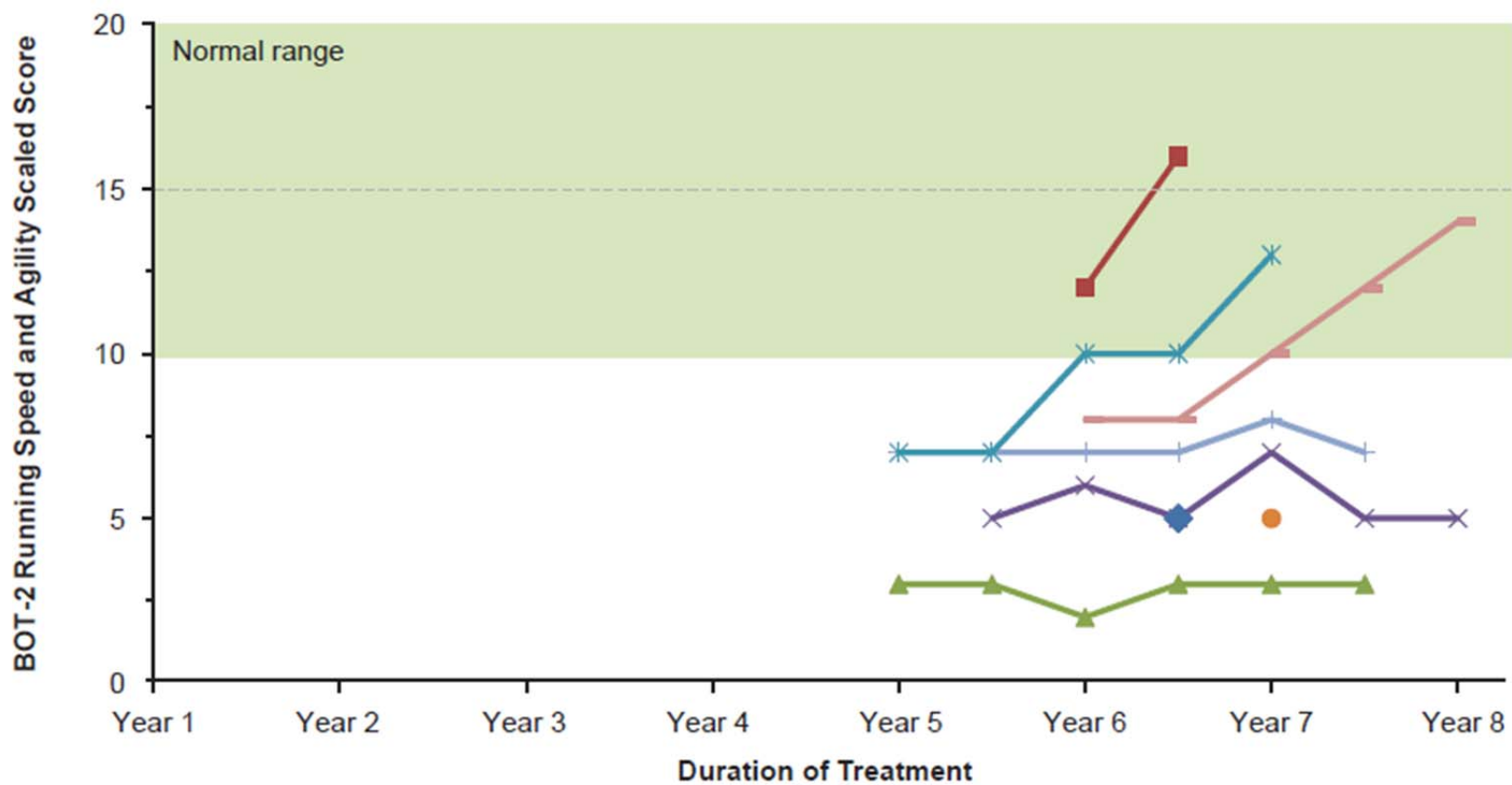
2

1 **Figure 6B**



2

1 Figure 6C



Patient ID (age [mo] at study entry):

—x— 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)

2