Asfotase Alfa Treatment Improves Survival for Perinatal and Infantile Hypophosphatasia

Michael P. Whyte, Cheryl Rockman-Greenberg, Keiichi Ozono, Richard Riese, Scott Moseley, Agustin Melian, David D. Thompson, Nicholas Bishop, and Christine Hofmann

Shriners Hospital for Children (M.P.W.) and Division of Bone and Mineral Diseases at Washington University School of Medicine (M.P.W.), St Louis, Missouri 63110; University of Manitoba and Children's Hospital Research Institute of Manitoba (C.R.-G.), Winnipeg, MB R3T 2N2 Canada; Graduate School of Medicine (K.O.), Osaka University, Osaka, 565-0871 Japan; Alexion Pharmaceuticals, Inc. (R.R., S.M., A.M., D.D.T.), Cheshire, Connecticut 06410; Department of Human Metabolism (N.B.), University of Sheffield, Sheffield, S10 2TN United Kingdom; Sheffield Children's Hospital (N.B.), Sheffield, S10 2TH United Kingdom; University Children's Hospital (C.H.), University of Würzburg, Würzburg, D-97080 Germany

Context: Hypophosphatasia (HPP) is an inborn error of metabolism that, in its most severe perinatal and infantile forms, results in 50–100% mortality, typically from respiratory complications.

Objectives: Our objective was to better understand the effect of treatment with asfotase alfa, a first-in-class enzyme replacement therapy, on mortality in neonates and infants with severe HPP.

Design/Setting: Data from patients with the perinatal and infantile forms of HPP in two ongoing, multicenter, multinational, open-label, phase 2 interventional studies of asfotase alfa treatment were compared with data from similar patients from a retrospective natural history study.

Patients: Thirty-seven treated patients (median treatment duration, 2.7 years) and 48 historical controls of similar chronological age and HPP characteristics.

Interventions: Treated patients received asfotase alfa as sc injections either 1 mg/kg six times per week or 2 mg/kg thrice weekly.

Main Outcome Measures: Survival, skeletal health quantified radiographically on treatment, and ventilatory status were the main outcome measures for this study.

Results: Asfotase alfa was associated with improved survival in treated patients vs historical controls: 95% vs 42% at age 1 year and 84% vs 27% at age 5 years, respectively ($P < .0001$, Kaplan-Meier log-rank test). Whereas 5% (1/20) of the historical controls who required ventilatory assistance survived, 76% (16/21) of the ventilated and treated patients survived, among whom 75% (12/16) were weaned from ventilatory support. This better respiratory outcome accompanied radiographic improvements in skeletal mineralization and health.

Conclusions: Asfotase alfa mineralizes the HPP skeleton, including the ribs, and improves respiratory function and survival in life-threatening perinatal and infantile HPP. **(*J Clin Endocrinol Metab* 101: 334–342, 2016)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received September 16, 2015. Accepted October 29, 2015.
First Published Online November 3, 2015

Abbreviations: ALP, alkaline phosphatase; CI, confidence interval; CPH, Cox proportional hazard; HC, historical control; HPP, hypophosphatasia; KM, Kaplan-Meier; RGI-C, Radiographic Global Impression of Change; TNSALP, tissue-nonspecific isoenzyme of ALP.

Hypophosphatasia (HPP) is the inborn error of metabolism that features low serum alkaline phosphatase (ALP) activity (hypophosphat*asemia*) caused by loss-of-function mutation(s) within the gene that encodes the tissue-nonspecific isoenzyme of ALP (TNSALP) (1, 2). TNSALP controls skeletal and dental mineralization by hydrolyzing inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal growth (3). Insufficient TNSALP activity in HPP can, therefore, lead to chest wall instability and respiratory complications.

HPP manifests remarkably broad-ranging expressivity (2) that is largely explained by its autosomal recessive and autosomal dominant patterns of inheritance involving at least 300 different mutations (predominantly missense) in the *TNSALP* (also called *ALPL*) gene (4–7). The clinical nosology for HPP organizes this expressivity by determining whether dental abnormalities are accompanied by skeletal disease and further complications, and, if so, the patient's age at HPP presentation (perinatal, infantile, childhood, adult, and odonto HPP) (8, 9). Perinatal HPP features extreme skeletal disease obvious at birth. Patients with infantile HPP develop substantial skeletal disease, failure to thrive, and sometimes vitamin B_6–dependent seizures postnatally before 6 months of age (9, 10). Perinatal HPP is almost always lethal near birth (11), whereas infantile HPP has an estimated 50% mortality during infancy (2), typically from respiratory complications (1, 2, 11). In both forms, hypomineralization leads to thoracic instability, fractures, and deformities, and sometimes, in perinatal HPP, to pulmonary hypoplasia (1, 12–14).

Asfotase alfa is the first-in-class, bone-targeted, enzyme-replacement therapy designed to reverse the skeletal mineralization defects in HPP. Pulmonary function improved during 1 year of treatment in an initial study of 11 infants and young children with life-threatening HPP (1). To better understand the effect of asfotase alfa treatment on mortality in neonates and infants with severe HPP, we evaluated survival, skeletal health, and ventilatory status from two studies of asfotase alfa treatment compared to historical outcomes for such patients.

Materials and Methods

Patients

Patients with perinatal or infantile HPP enrolled in two ongoing, multicenter, open-label, phase 2 interventional studies of asfotase alfa treatment (ENB-002-08 [NCT00744042]/ENB-003-08 [NCT01205152] and ENB-010-10 [NCT01176266]) as well as patients from one retrospective natural history study (ENB-011-10 [NCT01419028]) were eligible for inclusion in this comparative analysis of survival. Each study's protocol was approved by local institutional review boards and written informed consent was obtained from patients' parent(s) or legal guardian(s).

ENB-002-08/ENB-003-08

The methods for ENB-002-08/ENB-003-08, an open-label study that enrolled 11 patients with perinatal or infantile HPP to assess the safety, tolerability, and efficacy of asfotase alfa, are provided in detail elsewhere (1). Patients were enrolled at 12 sites worldwide from October 2008 to December 2009. These patients were 3 years of age or younger at enrollment, with symptoms of HPP before 6 months of age. All received asfotase alfa (40 mg/ml) as a single 2-mg/kg IV infusion followed by 1-mg/kg subcutaneous injections thrice weekly for 6 months (with dose increase up to 3 mg/kg thrice weekly if there was no radiographic evidence of skeletal improvement, deteriorating pulmonary function, or worsening failure to thrive), and then entered the ongoing extension phase. Respiratory support data recorded this status at semiannual evaluations. Scheduled radiographic assessments occurred at each 6-month study visit to evaluate the skeletal manifestations of HPP.

ENB-010-10

ENB-010-10 is an ongoing open-label study to further assess the safety, tolerability, and efficacy of asfotase alfa treatment for perinatal and infantile HPP (manuscript in preparation). Patients are 5 years of age or younger at enrollment with symptoms of HPP before age 6 months. In ENB-010-10, patients receive asfotase alfa as sc injection either 1 mg/kg six times per week or 2 mg/kg thrice weekly at the investigator's discretion (dose adjustments permitted in consultation with the sponsor). As in the ENB-002-08/ENB-003-08 study, therapeutic efficacy was assessed based on changes in the skeletal manifestations of HPP using radiographic evaluations at each semiannual study visit. Respiratory support data were also obtained for each visit. Patients were first enrolled in July 2010.

ENB-011-10

Because perinatal and infantile HPP are life-threatening, there was no placebo control group. Instead, we conducted a retrospective, multinational, noninterventional, natural history study (ENB-011-10; manuscript in preparation) of patients with perinatal or infantile HPP to provide a nonconcurrent historical control (HC) group for the two treatment studies. The principal inclusion criteria were a history of rachitic chest deformity, respiratory compromise, or vitamin B_6–dependent seizures, which were chosen to reflect the HPP pathophysiology and risk factors for mortality prevalent in the treated patients. Living and dead patient information from 12 sites in seven countries, including six sites in the United States and one site each in Australia, Canada, Germany, Spain, Taiwan, and Switzerland was acquired from September 2012 to April 2013. Data concerning the first 5 years of life were obtained from medical records by investigational site personnel.

Comparative analyses of treated patients vs HCs

Enrollment inclusion required signs or symptoms of HPP before 6 months of age. Patients in all three studies (ENB-002-08/ENB-003-08, ENB-010-10, and ENB-011-10) had been diagnosed with HPP and had one or more of the following: 1) *TNSALP* gene (*ALPL*) mutation; 2) serum ALP below the age-adjusted normal range and either plasma pyridoxal 5′-phos-

phate or urinary phosphoethanolamine above the upper limit of normal; and 3) serum ALP below the age-adjusted normal range and HPP-related radiographic abnormalities.

Comparative analyses further required one or more of the following recognized life-threatening complications in perinatal and infantile HPP: 1) respiratory compromise requiring supportive measures and/or medication and/or other pulmonary complications; 2) vitamin B_6–dependent seizures; and 3) rachitic chest deformity. These additional inclusion criteria were prospectively supported as risk factors by literature review (2, 14–18) and likely excluded patients with benign prenatal HPP (13, 19, 20).

Survival analyses

Kaplan-Meier (KM) analyses and Cox proportional hazards (CPH) regression models compared survival rates between treated patients and HCs and survival from first ventilation. Survival times were measured from birth to death and first ventilation to death, respectively. Sex was not considered a factor in the analysis of the data because of the small sample size. Living treated patients were censored at the time of their last recorded assessment. Living HCs were censored at the date of data abstraction. Patients whose status was unknown at the date of data abstraction were censored at the last known contact date.

Complete dates or partial or missing dates with recorded ages were used to derive the survival time for all events. If age was not available, missing months were imputed as "June" and missing days were imputed as "15." The KM log-rank test and the CPH Wald χ^2 test compared survival estimates between treated patients and HCs. Confidence intervals (CIs) for KM survival estimates were obtained using Greenwood's variance formula. For percentages of patients surviving, CIs for KM survival estimates were obtained using the normal approximation to the binomial distribution and KM plots were provided. CIs were also determined for the CPH hazard ratios. Superiority of asfotase alfa treatment over the HCs was established if the P value was $<.05$ and survival favored treatment.

All statistical analyses were conducted using SAS, version 9.2 (SAS Institute Inc.).

Relationship between skeletal health and respiratory function

To delineate the relationship between skeletal health and respiratory function, the clinical courses and outcomes of treated patients and HCs who required ventilatory support were assessed on a "by patient" basis. Ventilatory support was defined as "noninvasive" (ie, bilevel or continuous positive airway pressure, but not nasal oxygen or tracheostomy without mechanical ventilation) or "invasive" (ie, mechanical ventilation via endotracheal intubation or tracheostomy). In studies ENB-002-08/ENB-003-08 and ENB-010-10, two methods quantified skeletal manifestations of HPP assessed radiographically. A 10-point Rickets Severity Scale (10 = severe rickets, 0 = no rickets) quantified growth-plate abnormalities at the wrists and knees, evaluated by a single reader blinded to treatment time point (21), and reported here for baseline only. To assess changes from baseline in the skeletal health of the treated patients, paired sequential radiographs were rated for characteristic findings of HPP using the 7-point Radiographic Global Impression of Change (RGI-C) scale. A score of −3 indicates severe worsening; 0, no change; and +3, complete or nearly complete healing (1). For infants, raters examined wrist, knee, and chest radiographs for changes in metadiaphyseal patchy focal sclerosis, apparent physeal widening, irregularity of the provisional zone of calcification, metaphyseal radiolucencies, flaring, and/or fraying; thin, gracile bones; apparent absence of some or all bones; thin ribs; chest deformity; and evidence of recent fractures. Individual RGI-C scores were the mean across three raters blinded to treatment time point.

Results

Patients

A total of 65 patients with perinatal or infantile HPP were screened for the HC group (ENB-011-10) and 48 were enrolled. The asfotase alfa treatment group comprised 39 patients, 5 years of age or younger at enrollment, with perinatal or infantile HPP (11 patients from ENB-002-08/ENB-003-08 (1) and 28 patients from ENB-010-10). All patients in the analysis were enrolled before November 22, 2013, with interim data cut-offs of October 29, 2014, and November 12, 2014, respectively. Thirty-seven of the 39 patients enrolled in the treatment studies had a history of rachitic chest deformity, respiratory compromise, or vitamin B_6–dependent seizures, and were therefore compared with the HCs. All treated patients had two mutated *TNSALP* alleles.

Baseline demographic and clinical characteristics were similar for the two groups (Table 1). In both groups, most (>70%) patients had rachitic chest deformity and/or respiratory compromise, with fewer patients (<36%) having vitamin B_6–dependent seizures. Twenty-two percent (8/37) in the treated group and 17% (8/48) of the HCs had all three risk factors.

Survival

Asfotase alfa significantly improved survival ($P <$ .0001 based on KM log-rank test and $P = .0029$ based on CPH Wald χ^2 test) compared with the HCs (Figure 1). Ninety-five percent (35/37) of treated patients vs 42% (20/48) of HCs were alive at 1 year of age, and 84% (31/37) of treated patients vs 27% (13/48) of HCs were alive at 5 years of age (KM estimated survival 82% for treated patients). Median survival for the HCs was 8.9 months (95% CI, 5.1–14.0), but inestimable for the treated patients because most were living beyond the data cutoff.

Substantial mortality was observed for the HCs requiring ventilatory support and for those with vitamin B_6–dependent seizures, consistent with Baumgartner-Sigl et al, 2007 (10). Although these seizures can be temporarily managed with pyridoxine supplementation, no HCs with epilepsy survived (0%; 0/10). In contrast, survival was 77% (10/13) for the treated patients with seizures. The

Table 1. Baseline Demographic and Clinical Characteristics of Study Patients

Characteristic	Historical Controls[a]	Asfotase Alfa-Treated	*P* Value
	(N = 48)	(N = 37)	
Age enrolled (months)			
Mean ± SD	NA[b]	23 ± 24	
Median (minimum, maximum)	NA[b]	9 (0, 71)	
Age at HPP onset (months)	n = 47[c]	n = 35[d]	
Mean ± SD	1 ± 2	1 ± 2	
Median (minimum, maximum)	0.03 (0, 5.9)	1 (0, 5.8)	.80[e]
Age at HPP diagnosis (months)			
Mean ± SD	5 ± 9	NA[f]	
Median (minimum, maximum)	2 (0, 41)	NA[f]	
Sex, n (%)			.38[g]
Male	26 (54%)	16 (43%)	
Female	22 (46%)	21 (57%)	
Race, n (%)			.14[h]
White	40 (83%)	29 (78%)	
Asian	2 (4%)	6 (16%)	
Other	6 (13%)	2 (5%)	
Treatment duration (years)			
Median (minimum, maximum)	NA	2.70 (0.0, 6.0)	
Inclusion criteria, n (%)			
Rachitic chest[i]	40 (83%)	30 (81%)	.78[g]
Respiratory compromise[j]	40 (83%)	27 (73%)	.29[g]
Vitamin B_6–dependent seizures	10 (21%)	13 (35%)	.22[g]
All 3 features	8 (17%)	8 (22%)	.59[g]

Abbreviations: HPP, hypophosphatasia; NA, not applicable; SD, standard deviation.

[a] Year of birth: 1970s (n = 4; earliest 1970); 1980s (n = 9); 1990s (n = 14); 2000s (n = 15); 2010 and later (n = 6; youngest, August 2011).

[b] Data represent birth through age 5 years or death, whichever occurred first.

[c] A stillborn was excluded.

[d] The age at HPP onset was not available for 2 patients in study ENB-002-08/ENB-003-08 because of premature discontinuation from the study and closure of the sites after their discontinuation.

[e] Wilcoxon rank-sum test.

[f] Data not collected for treated patients.

[g] Fisher exact test.

[h] Pearson exact χ^2 test.

[i] In the retrospective study ENB-011-10 concerning the historical controls, a "rachitic chest" was identified from the available medical information.

[j] "Respiratory compromise" included decreased oxygen saturation, tachypnea, respiratory distress and/or failure, or other associated complications (eg, pneumonia, respiratory tract infection).

primary cause of death in the HCs, including those with seizures, was respiratory complications and/or failure.

Skeletal mineralization and respiratory function

Among the 48 HC patients, 20 required some form of ventilatory support (19/20 invasive) (Figure 2A). One survived, representing a 95% mortality rate. Figure 3 shows the outcomes for patients treated with asfotase alfa. Among the patients treated with asfotase alfa, there were also a large number (21/39) who required ventilator support: 14 at baseline and 7 soon after initiation of asfotase alfa treatment. Figure 2, B and C, show the respiratory outcomes and mortality of these treated patients who required ventilatory support at baseline.

Of all treated patients who required ventilatory support at baseline, 76% (16/21) survived, among whom 75% (12/16) were weaned from ventilatory support. One withdrew because of a reaction during the IV infusion that initiated treatment (1). Three died (1 of neurologic and respiratory complications, 1 of respiratory complications, and 1 of sepsis associated with pneumonia). In the 10 remaining treated patients, 7 were weaned from all ventilatory support and 1 advanced from invasive to noninvasive ventilation. Two patients still required invasive ventilation at the last visit, although inspiratory pressure requirements were lower. In the 7 patients who initiated ventilatory support postbaseline (Figure 2C), there were 2 deaths: 1 from pneumonia and 1 from neurologic complications of craniosynostosis. The remaining 5 patients were weaned from ventilatory support. Generally, the ventilatory support for these patients was required early dur-

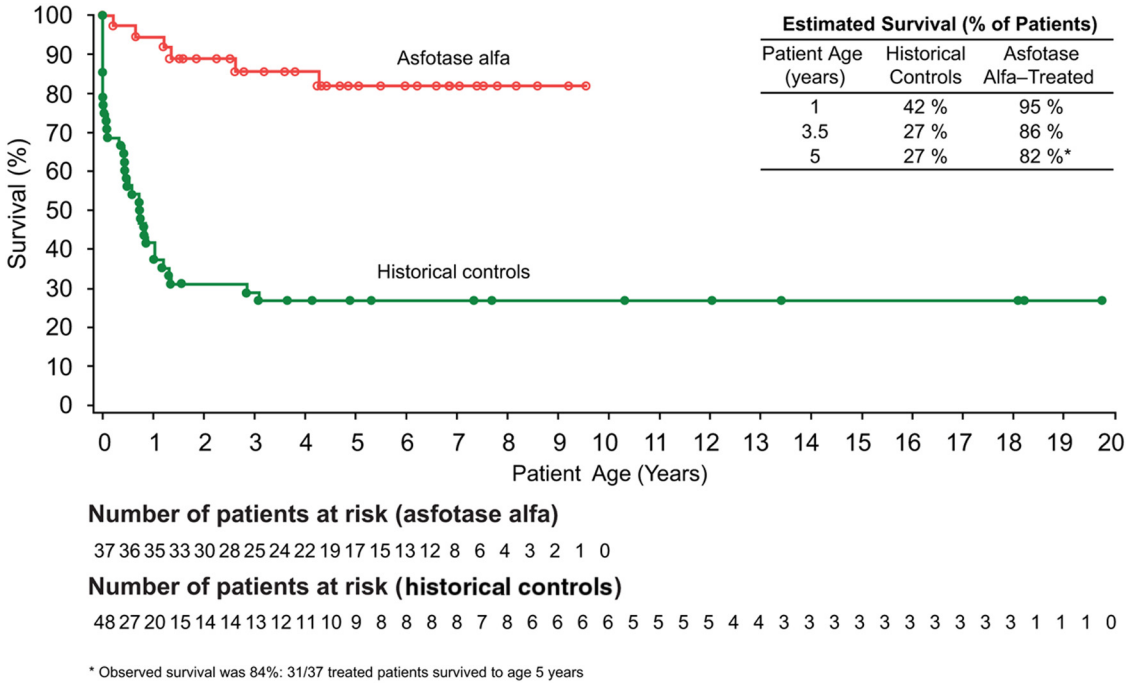

Figure 1. Kaplan-Meier analysis of survival of patients with perinatal and infantile hypophosphatasia. Asfotase alfa–treated patients (studies ENB-002-08/ENB-003-08 and ENB-010-10) had a significantly better survival rate, calculated using the Kaplan-Meier product-limit estimate, compared with historical controls (study ENB-011-10; $P < .0001$, Kaplan-Meier log-rank test).

ing treatment before there were substantial improvements in bone mineralization.

Typically, improved ventilation among the treated patients was associated with, and preceded by, better skeletal mineralization documented by the RGI-C scores. All 12 patients who were weaned from ventilatory support had, or would soon achieve, an RGI-C score of at least +2, indicating substantial improvement in HPP-related skeletal abnormalities. None, once weaned, required further ventilatory support. Representative radiographs with concurrent ventilatory status are shown in Figure 4.

Discussion

Our results demonstrate that asfotase alfa treatment is associated with improved mineralization of the skeleton, including the ribs, improved respiratory function, and improved survival in patients with perinatal and infantile HPP. Consistent with a true treatment effect, improved survival was greatest in patient subgroups at the greatest risk of death, specifically patients with vitamin B_6–responsive seizures and those requiring ventilatory support.

Our findings document that improved respiratory function accompanies better skeletal mineralization and modeling in these asfotase alfa–treated patients. Respiratory failure in patients with perinatal or infantile HPP can be complex and include thoracic deformity and fractures, pulmonary hypoplasia, muscle weakness, tracheomalacia, central nervous system dysfunction associated with craniosynostosis, episodic seizures, and perhaps predisposition to infection because of low TNSALP levels in leukocytes (2, 10, 13, 14, 22–27). In some asfotase alfa–treated patients, respiratory deterioration occurred early during treatment, possibly reflecting the disorder's natural history and complications. However, once sufficient time for improvements in mineralization had occurred (as measured by RGI-C), most patients were able to come off of mechanical ventilation, consistent with stabilization of the chest wall and/or improved muscle strength. Hence, ventilator and intensive supportive care may be necessary during the early months of asfotase alfa treatment, but with continued treatment, most infants are able to be weaned from mechanical ventilation. In some patients, respiratory status improved less rapidly, perhaps reflecting severe or long-standing pulmonary impairment at baseline. These patients may have benefited from earlier intervention.

Although the data from the HC group spanned three decades (Figure 2A), advances in supportive respiratory care are unlikely to explain the better outcomes of the contemporary treatment group. Several major improvements in the respiratory care of critically ill infants occurred approximately from 1980 to 1990, including recognition of oxygen toxicity, use of surfactant replacement therapy, and high-frequency ventilation (28–32). Furthermore, strategies for noninvasive ventilation advanced after the early 2000s (33, 34). However, the outcomes of

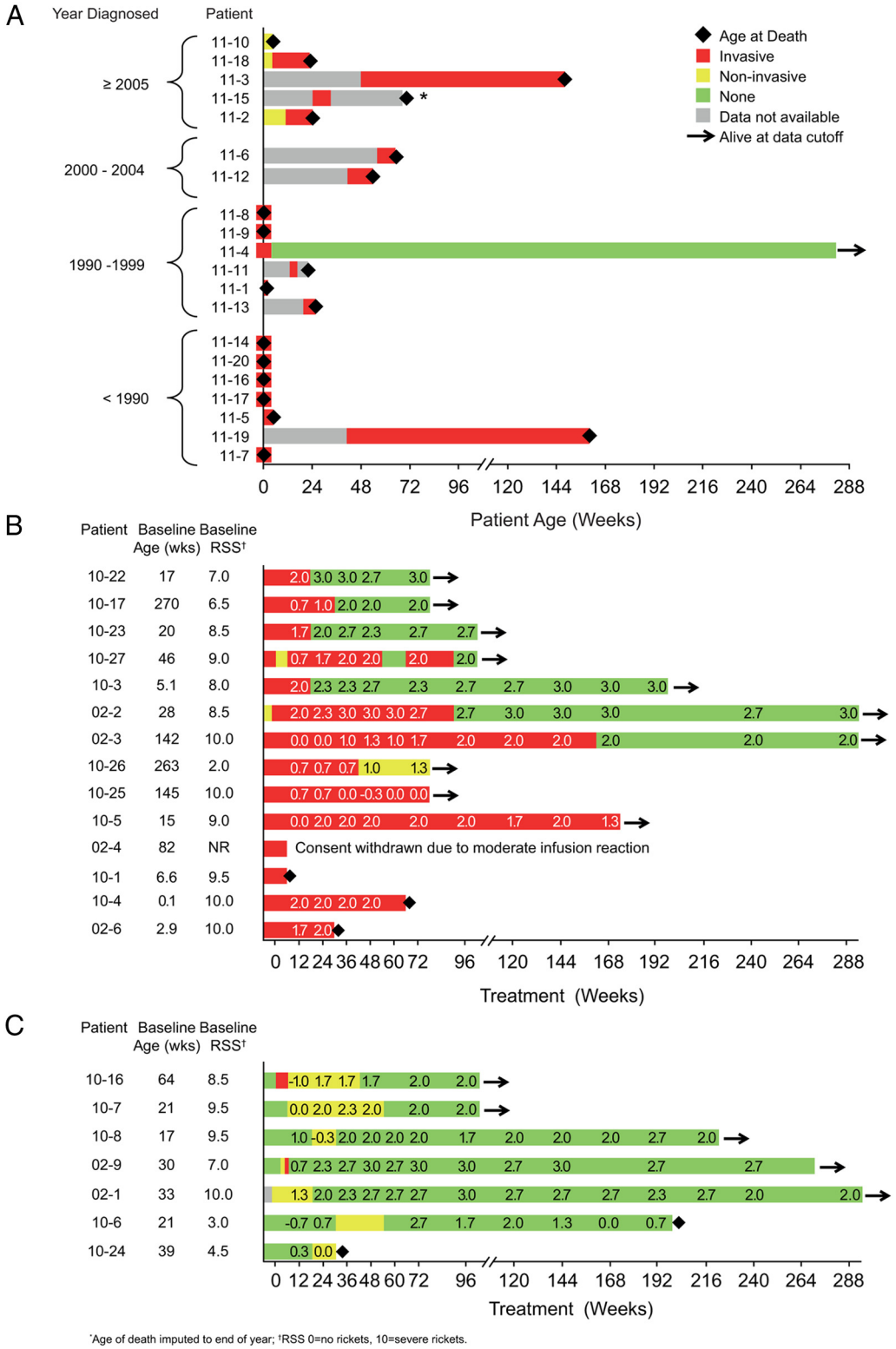

Figure 2. Ventilatory support and patient outcomes in asfotase alfa–treated and historical control patients. (A) Ventilatory status and patient outcome for the 20 historical control patients (study ENB-011-10) who required ventilatory support in the first 5 years of life. (B) Ventilatory support status improved with bone mineralization for asfotase alfa–treated patients who required ventilatory support at baseline (n = 14) or (C) did not require ventilatory support at baseline but required support later in the study (n = 7). Initiation and discontinuation of ventilatory support are shown as colored bars. Death is indicated by black diamonds. Patient number, age at enrollment in studies ENB-002-08/ENB-003-08 (patients numbered according to Whyte et al (1)) and ENB-010-10, and baseline Rickets Severity Score (RSS); 0 = no rickets, 10 = severe rickets (21)) are shown on the left. Numbers within the colored bars represent the Radiographic Global Impression of Change (RGI-C) scale score. NR, not recorded.

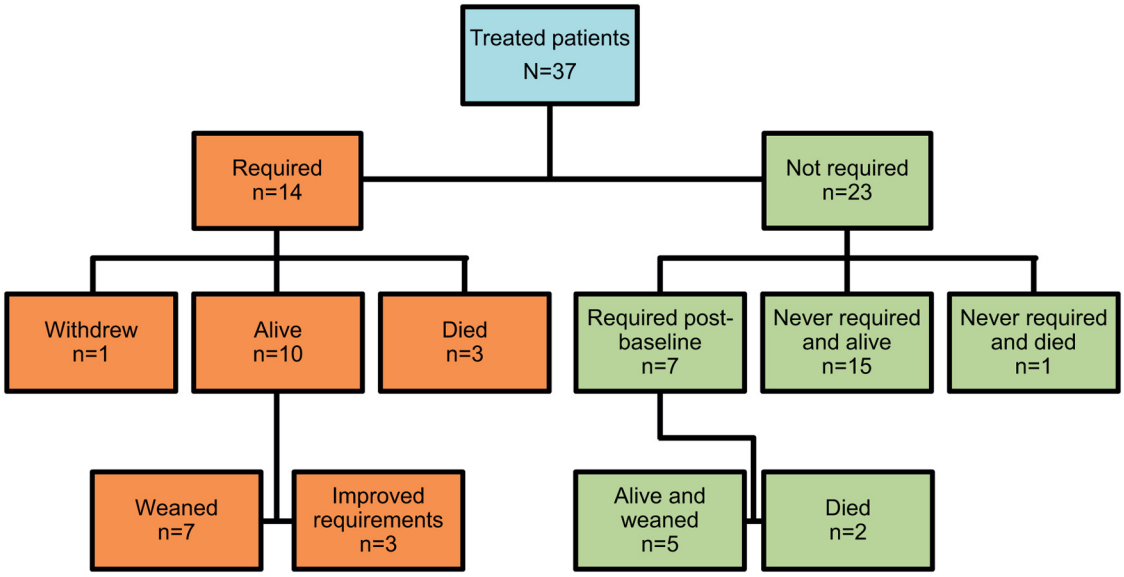

Figure 3. Patient outcomes according to ventilatory status at baseline. Ventilation includes both noninvasive and invasive (see Materials and Methods).

noninvasive ventilation strategies for chronic, progressive, debilitating diseases, such as infantile HPP can be, are not well-established, and these strategies are unlikely to significantly impact mortality in untreated HPP. Notably, although the 7 HC patients diagnosed in the year 2000 or later tended to survive longer on ventilatory support, they all eventually died (Figure 2A), indicating that supportive care alone cannot overcome perinatal or infantile HPP.

The better survival of patients with HPP given asfotase alfa is presumably from improved skeletal mineralization and structure that stabilizes the chest, permitting weaning from mechanical ventilation and fewer respiratory events. Evidence for stabilization of the chest wall and improvement in respiratory mechanics with asfotase alfa was described in detail for 1 patient with perinatal HPP (Patient 02–6) (1, 14), who first received asfotase alfa treatment at 2.9 weeks of age. During the following 12 weeks, this boy had a progressive decrease in ventilator requirements accompanying improvements in chest wall mechanics and lung function that correlated with radiographic improvements in skeletal mineralization. Most noticeably, he improved from a highly paradoxical breathing pattern to a more normal, synchronous pattern during treatment (14).

These cumulative findings support and significantly expand the published 1-year observations from the initial uncontrolled cohort of 11 treated patients (1), now with up to 6 years of treatment, by adding 48 matched HCs and a total of 37 treated patients. Benefits were generally noted

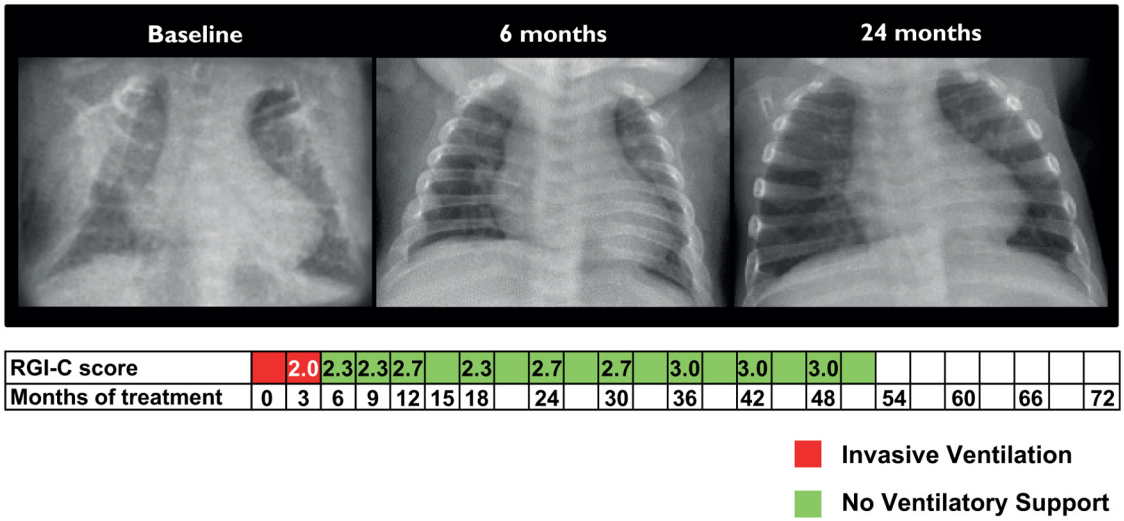

Figure 4. Radiographic changes with asfotase alfa treatment (Patient 10–3). Better rib mineralization, chest structure, and thoracic volume with improved ventilatory status in an infant with hypophosphatasia (5.1 weeks of age at treatment baseline). RGI-C, Radiographic Global Impression of Change scale.

regardless of baseline severity characteristics (rachitic chest, seizures, or respiratory insufficiency), including patients with founder mutations (eg, c.1001G>A, Canadian Mennonites (35); c.1559delT, Japan (36, 37)).

These results provide important information regarding the expected outcomes with asfotase alfa treatment for infants and young children with perinatal and infantile HPP. Compared with the poor outcomes of HCs receiving supportive care, asfotase alfa treatment substantially improved survival for patients with life-threatening perinatal and infantile HPP. Increased skeletal mineralization was associated with better respiratory outcomes, ultimately conferring the survival benefit.

Acknowledgments

The authors thank the parents and patients for making this study possible.

The following investigators were collaborators and could comment on the draft paper. ENB-002-08/ENB-003-08: Richard E. Lutz, University of Nebraska Medical Center, Omaha, NE; Mairead McGinn, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK; Nada J. Salman, Tawam Hospital, Al Ain, United Arab Emirates; Jill H. Simmons, Vanderbilt University, Nashville, TN; John W. Taylor, Prevea Health Clinic and St Vincent's Hospital, Green Bay, WI. ENB-011-10: Linda DiMeglio, Indiana University School of Medicine, Indianapolis, IN; Wuh-Liang Hwu, National Taiwan University Hospital, Taipei, Taiwan; Edward Leung and Aizeddin Mhanni, University of Manitoba, Winnipeg, Manitoba, Canada; Gabriel Á. Martos-Moreno and Jesús Argente, Department of Endocrinology, Hospital Infantil Universitario Niño Jesús. Department of Pediatrics, Universidad Autónoma de Madrid. CIBEROBN, ISCIII. Madrid, Spain; Peter Simm, The Royal Children's Hospital Melbourne and Murdoch Children's Research Institute, Melbourne, Australia; Robert Steiner, University of Wisconsin School of Medicine and Public Health, Madison, WI; Andreas Superti-Furga, Universitätsklinikum Würzburg Kinderklinik, Pädiatrische Infektiologie und Immunologie, Würzburg, Germany; William Wilcox, Cedars-Sinai Medical Center, Los Angeles, CA. ENB-010-10: Paul Harmatz, University of California San Francisco Benioff Children's Hospital Oakland, Oakland, CA; Seiki Horita, Ishikawa Prefectural Central Hospital, Japan; Wuh-Liang Hwu, National Taiwan University Hospital, Taiwan; Hirashi Kawashima, Tokyo Medical University Hospital, Japan; Hiroshi Masaki, St Marianna University School of Medicine, Japan; Hideki Nakayama, National Hospital Organization Fukuoka Higashi Medical Center, Japan; Halil Saglam, Uludag University Faculty of Medicine, Turkey; Seiji Sato, Saitama Municipal Hospital, Japan; and Gerald Vockley, Children's Hospital of Pittsburgh, PA.

Eileen Sawyer of Alexion Pharmaceuticals helped create, edit, and finalize the manuscript. Johannes Liese of Würzburg, Germany, and Katherine L. Madson of St Louis, MO, gave critical review and helpful comments. Amy Reeves of St Louis, MO, assisted in chart reviews. Sarah Thornburg of Fishawack Communications GmbH and Sharon McKenzie of St Louis, MO, helped prepare the report.

Address all correspondence and requests for reprints to: Michael P. Whyte, MD, Shriners Hospital for Children, 4400 Clayton Ave., St Louis, MO 63110. E-mail: mwhyte@shrinenet.org.

This study was funded by Alexion Pharmaceuticals, Inc. Partial funding for this study was provided by a grant (FD-R-003745-03) from the Office of Orphan Products Development (OOPD). Funding for the University of California San Francisco Benioff Children's Hospital Oakland's ENB-010-10 segment was derived in part through University of California San Francisco-Clinical & Translational Science Institute Grant UL1 TR000004. Work done by Fishawack Communications GmbH, Basel, Switzerland, in preparing the report was funded by Alexion Pharmaceuticals, Inc.

Presented in part at the Pediatric Academic Societies 2014 Annual Meeting, Vancouver, BC, Canada, May 3–6, 2014 (Whyte et al, available at: http://www.abstracts2view.com/pasall/view.php?nu=PAS14L1_3808.200); the 28th Annual Meeting, American Society for Bone and Mineral Research, Houston, TX, September 12–15, 2014 (Whyte et al, J Bone Miner Res 29 (Suppl 1); and the American College of Medical Genetics and Genomics 2015 Annual Clinical Genetics Meeting, Salt Lake City, UT, March 24–28, 2015 (Leung et al, available at: https://ww4.aievolution.com/acm1501/index.cfm?do=abs.viewAbs&abs=1389).

Clinical Trial Registration Numbers: NCT00744042, NCT01205152, NCT01176266, and NCT01419028. Contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Author Contributions: All authors designed the studies, contributed to data interpretation, and critically revised and approved the submitted manuscript. M.P.W., C.R.-G., N.B., and C.H. treated patients. S.M. and R.R. analyzed data. M.P.W. and R.R. drafted the manuscript.

Disclosure Summary: M.P.W., C.R.-G., N.B., and C.H. are study investigators with Alexion Pharmaceuticals, Inc. M.P.W. and C.H. received research grants; M.P.W., C.R.-G., N.B., and C.H. received honoraria and travel support; and K.O. and C.H. received consultancy fees from Alexion Pharmaceuticals, Inc. R.R., S.M., A.M., and D.D.T. are employees of Alexion Pharmaceuticals, Inc.

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