RESEARCH ARTICLE

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Safety and Physiological Effects of Two Different Doses of Elosulfase Alfa in Patients With Morquio A Syndrome: A Randomized, Double-blind, Pilot Study

Barbara K. Burton,[1,2] Kenneth I. Berger,[3] Gregory D. Lewis,[4] Mark Tarnopolsky,[5] Marsha Treadwell,[6] John J. Mitchell,[7] Nicole Muschol,[8] Simon A. Jones,[9] V. Reid Sutton,[10,11] Gregory M. Pastores,[3,12] Heather Lau,[3] Rebecca Sparkes,[13] Fred Genter,[14] Adam J. Shaywitz,[14] and Paul Harmatz[6]*

[1]Ann and Robert H. Lurie Children's Hospital, Chicago, Illinois

[2]Northwestern University Feinberg School of Medicine, Chicago, Illinois

[3]New York University School of Medicine, New York, New York

[4]Massachusetts General Hospital, Boston, Massachusetts

[5]McMaster University Medical Center, Hamilton, Ontario, Canada

[6]UCSF Benioff Children's Hospital Oakland, Oakland, California

[7]Montreal Children's Hospital, Montreal, Quebec, Canada

[8]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[9]Willink Unit, St. Mary's Hospital, CMFT, MAHSC, University of Manchester, Manchester, United Kingdom

[10]Texas Children's Hospital, Houston, Texas

[11]Baylor College of Medicine, Houston, Texas

[12]Mater Misericordiae University Hospital, Dublin, Ireland

[13]Alberta Children's Hospital, Calgary, Alberta, Canada

[14]BioMarin Pharmaceutical Inc., Novato, California

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The primary treatment outcomes of a phase 2, randomized, double-blind, pilot study evaluating safety, physiological, and pharmacological effects of elosulfase alfa in patients with Morquio A syndrome are herewith presented. Patients aged ≥7 years and able to walk ≥200 m in the 6-min walk test (6MWT) were randomized to elosulfase alfa 2.0 or 4.0 mg/kg/week for 27 weeks. The primary objective was to evaluate the safety of both doses. Secondary objectives were to evaluate effects on endurance (6MWT and 3-min stair climb test [3MSCT]), exercise capacity (cardio-pulmonary exercise test [CPET]), respiratory function, muscle strength, cardiac function, pain, and urine keratan sulfate (uKS) levels, and to determine pharmacokinetic param-

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*Correspondence to:
Paul Harmatz, UCSF Benioff Children's Hospital Oakland, 747 52nd St.Oakland, CA 94609
Email: pharmatz@mail.cho.org

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eters. Twenty-five patients were enrolled (15 randomized to 2.0 mg/kg/week and 10 to 4.0 mg/kg/week). No new or unexpected safety signals were observed. After 24 weeks, there were no improvements versus baseline in the 6MWT, yet numerical improvements were seen in the 3MSCT with 4.0 mg/kg/week. uKS and pharmacokinetic data suggested no linear relationship over the 2.0–4.0 mg/kg dose range. Overall, an abnormal exercise capacity (evaluated in 10 and 5 patients in the 2.0 and 4.0 mg/kg/week groups, respectively), impaired muscle strength, and considerable pain were observed at baseline, and there were trends towards improvements in all domains after treatment. In conclusion, preliminary data of this small study in a Morquio A population with relatively good endurance confirmed the acceptable safety profile of elosulfase alfa and showed a trend of increased exercise capacity and muscle strength and decreased pain.

Key words: mucopolysaccharidosis IV; cardiopulmonary exercise test; safety; respiratory function tests; muscle strength; physical endurance; GALNS protein; human [supplementary concept]; enzyme replacement therapy

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INTRODUCTION

Morquio A syndrome, also known as mucopolysaccharidosis (MPS) IVA, is a lysosomal storage disorder occurring in 1 per 640,000 to 1 per 76,000 live births, depending on the country of origin [Tomatsu et al., 2011]. Due to a deficiency in the glycosaminoglycan (GAG)-degrading enzyme N-acetylgalactosamine-6-sulfatase (GALNS), undegraded and partially degraded keratan sulfate (KS) and chondroitin-6-sulfate accumulate in multiple tissues and organs of Morquio A patients [Yasuda et al., 2013]. This accumulation causes cellular and organ dysfunction leading to the progressive development of an array of clinical manifestations. Most apparent are the musculoskeletal and joint issues, including short-trunk dwarfism and chest, spine, hip, knee, and ankle abnormalities, atlantoaxial instability, and wrist hypermobility [Harmatz et al., 2013; Hendriksz et al., 2015a]. Common non-skeletal manifestations include cardiorespiratory compromise, spinal cord compression, corneal clouding, hearing loss, hepatosplenomegaly, and dental abnormalities [Harmatz et al., 2013; Hendriksz et al., 2013; Hendriksz et al., 2015a]. Most patients with Morquio A syndrome show reduced endurance and/or mobility [Harmatz et al., 2013], which can be due to musculoskeletal abnormalities, joint pain, cardiorespiratory compromise, and/or neurological disease secondary to spinal cord compression.

The only approved disease-modifying therapy currently available for Morquio A syndrome is enzyme replacement therapy (ERT) with elosulfase alfa (VIMIZIM®, BioMarin Pharmaceutical Inc., Novato). Several studies provided consistent evidence of clinically meaningful and sustained improvement in health and function of Morquio A patients treated with elosulfase alfa at 2.0 mg/kg/week as assessed by measures of endurance (e.g., 6-min walk test [6MWT]), respiratory function, growth, and quality of life [Hendriksz et al., 2012; Hendriksz et al., 2014a; Jones et al., 2015]. In the pivotal phase 3, double-blind, placebo-controlled study including 176 Morquio A patients with impaired endurance/mobility, elosulfase alfa showed an acceptable safety profile, comparable with ERTs for other MPS disorders, and a statistically significant impact on walking distance in the 6MWT after 24 weeks of treatment. No significant impact was seen in the 3-min stair climb test (3MSCT). In addition, a rapid and sustained reduction in urine KS (uKS) levels and numerical improvements in several other efficacy outcomes, including respiratory function and growth, were observed [Hendriksz et al., 2014a; Hendriksz et al., 2015b].

In this report, we present results from the 27-week primary treatment phase of an ongoing study evaluating the safety of two dose levels, 2.0 and 4.0 mg/kg/week, of elosulfase alfa in patients with Morquio A syndrome with a relatively good endurance level/mobility (required to walk $\geq$200 m at baseline in the 6MWT). The study evaluated effects on endurance in the 6MWT and 3MSCT and determined pharmacokinetic parameters and impact on uKS levels of the two doses. In order to obtain new insights into the physiology and symptomatology of Morquio A syndrome, exercise capacity and various possible physiological contributors to endurance such as cardiac function, respiratory function, and pain in this study population were assessed as well.

MATERIALS AND METHODS

Study Design and Patient Selection

We present results of the primary treatment component of an ongoing phase 2, two-arm, randomized, double-blind, pilot study that is being conducted by 8 principal investigators at 8 centers in 4 countries (Germany, United Kingdom, Canada, United States). The planned study duration is up to 196 weeks, including a 3-week screening, a 27-week primary treatment phase, and an up to 166-week extension phase. While safety was assessed throughout the duration of the study, efficacy endpoints were assessed over 24 or 25 weeks.

Subjects with a documented diagnosis of Morquio A syndrome who were at least 7 years of age, were able to walk at least 200 m in the 6MWT at screening, and had not previously had a hematopoietic stem cell transplant or been previously treated with elosulfase alfa were eligible to participate in the study. Patients with severe untreated sleep apnea (as measured by a home sleep testing device), a requirement for supplemental oxygen or ventilation, or any medical condition, including but not limited to symptomatic

cervical spine instability or cord compression, that would interfere with study participation as determined by the investigator, were excluded by the protocol. A 6MWT distance of ≥200 m and age ≥7 years were selected as inclusion criteria in order to enroll a population of subjects who were able to perform a cardio-pulmonary exercise test (CPET) of sufficient duration to provide useful information on cardio-pulmonary/exercise capacity.

Randomization was stratified by cohort (A or B). The 15 subjects enrolled in cohort A were randomized 2:1 to receive 2.0 or 4.0 mg/kg/week elosulfase alfa and performed all study procedures, including a CPET. After completion of enrollment in cohort A, 10 additional subjects were enrolled in cohort B and randomized 1:1 to receive 2.0 or 4.0 mg/kg/week elosulfase alfa; cohort B subjects performed all study procedures except for a CPET. The number of subjects required to perform a CPET was limited to 15 due to the complexity of the procedure. The dose of 4.0 mg/kg/week was selected based on the results of the phase 1/2 clinical study and in vitro studies of K_{uptake} and lysosomal clearance. Nonclinical safety studies evaluating anaphylactoid-type reactions, formation of anti-elosulfase alfa antibodies (Abs), adverse events (AEs), and developmental and reproductive toxicology in rats, rabbits, and monkeys supported treatment with a dose up to 4.0 mg/kg/week (data on file, BioMarin). The primary objective of the primary treatment period was to evaluate the safety of 2.0 and 4.0 mg/kg/week doses of elosulfase alfa. The secondary objectives were to evaluate the effect of both doses on endurance (in a 6MWT and 3MSCT), overall exercise capacity, respiratory function, muscle strength, cardiac function, pain, and uKS levels and to determine the pharmacokinetic variables of elosulfase alfa.

All patients were pretreated with an appropriate dose of antihistamine medication, with or without antipyretic medications, approximately 30 min before the infusion in order to reduce the risk of hypersensitivity reactions. The study drug was infused intravenously to deliver the total infusion volume of either 250 or 400 ml (depending on the subject's weight) over a period of approximately 4 h. Treatment assignment was unknown to study subjects, investigators, site personnel, and BioMarin (the sponsor of the study).

The research was prospectively reviewed and approved by the duly constituted Institutional Review Board, Independent Ethics Committee, or Research Ethics Board at each participating center.

Safety Evaluation

Safety was assessed throughout the study period by evaluating AEs, clinical laboratory assessments, vital signs, physical examinations, electrocardiograms (ECG), echocardiography, immunogenicity, and pregnancy testing. Severity, seriousness, and relationship to study drug were determined using the NCI CTCAE v4. Potential hypersensitivity AEs were identified by utilizing the broad Anaphylactic Reaction algorithmic Standardized MedDRA query and the broad Angioedema Standardized MedDRA query. An infusion associated reaction (IAR) was defined as any AE, including anaphylaxis, anaphylactoid reactions, and other allergic reactions, occurring after the onset of the infusion and within 1 day following the end of the infusion, regardless of relationship to study drug.

Serum samples for immunogenicity testing were collected prior to dose administration at baseline and at weeks 2, 4, 6, 12, and 24. Routine immunogenicity testing included validated assays for anti-elosulfase alfa total antibody (TAb) and anti-drug Abs that inhibit binding to the mannose-6-phosphate receptor (neutralizing Abs; NAb) [Schweighardt B et al., 2014]. Anti-drug IgE, C4 and serum tryptase were assessed when patients experienced a severe IAR or an IAR requiring infusion cessation.

Efficacy Evaluation

Appendix 1 (See Supporting Information Online) provides a schedule of the secondary efficacy and pharmacokinetic assessments performed during the primary treatment phase.

Endurance was measured in duplicate (7-day time window, one test/day) at each time point by the 6MWT [American Thoracic Society, 2002] and the 3MSCT. The outcome of the tests was the average of duplicate test results. Overall exercise capacity was measured using CPET (cohort A only) and performed on a separate day and after completion of the two endurance tests. Patients performed maximal incremental exercise testing using an electronically braked upright cycle ergometer. Expired oxygen and CO_2 were analyzed via an expired gas analysis system, heart rate was monitored by continuous 3- or 12-lead ECG, and oxygen saturation was measured via pulse oximetry. Exercise capacity was assessed by both the peak workload and peak VO_2 (volume of oxygen uptake) achieved during the CPET. All CPET data were interpreted centrally in a core laboratory. Respiratory function (forced vital capacity, forced expiratory volume in 1 s) was assessed by spirometry in accordance with the American Thoracic Society Standards [Miller et al., 2005]. A home sleep testing device was used to assess the presence and severity of sleep-disordered breathing by measurement of blood oxygen saturation, pulse rate, and airflow during overnight monitoring. Muscle strength (knee extension, knee flexion, elbow flexion) was measured on a different day from the 6MWT, 3MSCT, or the CPET using an isokinetic dynamometer. Each test was conducted in triplicate and the maximum of the triplicate observations was used in the analysis. Cardiac function was evaluated by a standard 2-dimensional Doppler echocardiogram at screening and at week 24 and assessed centrally. Pain was measured using the Adolescent Pediatric Pain Tool (APPT), a validated multidimensional tool to evaluate pain in children, adolescents, and young adults [Jacob et al., 2014]. Within the APPT, overall pain intensity was evaluated by a Word Graphic Rating Scale (WGRS), a 10-cm visual analog scale ranging from "no pain" (0 cm) to "worst possible pain" (10 cm). Subjects indicated pain location on a body diagram. uKS was measured by liquid chromatography tandem mass spectroscopy and normalized using urinary creatinine [Martell et al., 2011]. Blood samples for pharmacokinetic analysis were obtained at weeks 0 and 23 within 15 min prior to dosing, 60 and 120 min after the start of the infusion, at the end of the infusion, and 5, 15, 30, 60, 120, and 180 min post-infusion.

Enzyme tests were performed in different laboratories using different testing protocols and were therefore not comparable. The enzyme activity levels required for study entry had to be below a cutoff and were difficult to compare in this range. Basic GALNS genotyping was not required, but was done in all patients. However, sophisticated techniques to differentiate heterozygous mutations were not performed. Given these constraints and the small sample

size of the study, there were no pre-specified analyses comparing genotype or enzyme activity levels to study endpoints.

Statistical Methods

The screening assessment values were used as baseline values. Baseline summaries and safety analysis are descriptive. The safety analysis included all patients who received any amount of elosulfase alfa during the study. Dosing compliance was derived from the total amount of study drug intake divided by the planned study drug intake over the study period, and multiplied by 100%.

Efficacy analyses include descriptive statistics for all secondary efficacy variables. The analyses for all efficacy endpoints were based on the modified intent-to-treat (MITT) population, consisting of all subjects who were randomized to study treatment, received at least one dose of study drug, and had at least one post-treatment observation. The relationship between immunogenicity and uKS levels was assessed by linear regression analysis (uKS vs. TAb titers and NAb positivity rates at week 24) and by plotting the mean percent change in normalized uKS against visit week, by overall mean TAb titer group ($\leq$ or $>$ the overall mean TAb titer) and by NAb positivity ($\leq$50% positive, $>$50% positive) for each treatment group.

Pharmacokinetic parameters of elosulfase alfa were calculated for the 2.0 and 4.0 mg/kg/week dose groups at weeks 0 and 23 by standard noncompartmental analysis according to current working practices and using WinNonlin version 6.1 [Shargel et al., 2005]. Actual sampling times and infusion duration were used in the pharmacokinetic calculations as there were some out of the $\pm$5% range of the nominal ones for some subjects. Because of the short elosulfase alfa half-life (mean $t_{1/2}$ $<$1 h) relative to dosing interval (1 week), each infusion was treated as a single dose for pharmacokinetic analysis.

RESULTS

Patient Characteristics

Twenty-five patients were enrolled in the study, including 15 randomized to elosulfase alfa 2.0 mg/kg/week and 10 to elosulfase alfa 4.0 mg/kg/week. Baseline characteristics are shown in Table I. Median age was 11.5 years, with only three patients (in the 2.0 mg/kg/week group) who were older than 18 years. Patients showed better endurance in the 6MWT and 3MSCT (Table I) than the subjects assessed in the previous phase 3 study in Morquio A in which the entry criterion was 6MWT distance $\geq$30 and $\leq$325 m during screening (mean 6MWT distance 372.2 m in this study vs. 203.9–211.9 m in the phase 3 study; mean 3MSCT result 65.0 stairs/min in this study, vs. 27.1–30.0 stairs/min in the phase 3 study) [Hendriksz et al., 2014a]. Only one patient in this study, in the 2.0 mg/kg/week group, used a walking aid during the test. The majority (80%) of patients showed short stature (height below 3rd percentile), with a mean height of 119.9 cm. The most commonly reported medical history findings by preferred term were arthralgia (40%), body height below normal for age (26.7%), hip dysplasia (26.7%), pectus carinatum (26.7%), joint laxity (26.7%), and corneal opacity (26.7%) in the elosulfase alfa 2.0 mg/kg/week group and arthralgia (60%), knee deformity (50%), enamel anomaly (40%), medical device implantation (40%), and medical device removal (40%) in the elosulfase alfa 4.0 mg/kg/week group. All patients in both treatment groups completed the study.

TABLE I. Patient Demographics and Baseline Characteristics in Elosulfase Alfa 2.0 and 4.0 mg/kg/week Dose Groups and in the Whole Study Group (Total)

	2.0 mg/kg/week N = 15	4.0 mg/kg/week N = 10	Total N = 25
Age at enrollment (years)			
Mean (SD)	14.9 (9.32)	12.0 (3.16)	13.7 (7.5)
Median (range)	11.3 (7.5, 39.5)	12.2 (7.8, 17.6)	11.5 (7.8, 39.5)
Sex, n (%)			
Female	12 (80.0)	4 (40.0)	16 (64.0)
Male	3 (20.0)	6 (60.0)	9 (36.0)
Race, n (%)			
White	14 (93.3)	9 (90.0)	23 (92.0)
Other	1 (6.7)	1 (10)	2 (8.0)
Height percentile, n (%)			
<3rd percentile	11 (73.3)	9 (90.0)	20 (80.0)
≥3rd percentile	4 (26.7)	1 (10.0)	5 (20.0)
6MWT, m			
Mean (SD)	369.6 (89.2)	376.3 (70.0)	372.2 (80.6)
Median (range)	346.8 (255, 596)	393.2 (267, 453)	372.3 (255, 596)
3MSCT, stairs/min			
Mean (SD)	65.5 (21.4)	64.2 (23.3)	65.0 (21.7)
Median (range)	65.3 (28, 119)	63.6 (30, 100)	65.2 (28, 119)
uKS, μg/mg			
Mean (SD)	16.4 (15.2)	18.8 (9.0)	17.4 (13.0)
Median (range)	13.0 (1.8, 52.4)	17.2 (7.0, 33.3)	13.2 (1.8, 52.4)

uKS: N = 14 in 2.0 mg/kg/week and N = 9 in 4.0 mg/kg/week groups.

TABLE II. Patients Experiencing Adverse Events (AEs) and Infusion Interruptions Due to AEs in the Elosulfase Alfa 2.0 and 4.0 mg/kg/week Dose Groups and in the Total Group

	2.0 mg/kg/week N = 15	4.0 mg/kg/week N = 10	Total N = 25
Any AE	15 (100.0%)	10 (100.0%)	25 (100%)
Drug-related AE	14 (93.3%)	8 (80.0%)	22 (88.0%)
Any SAE	0	1 (10.0%)	1 (4.0%)
Hypersensitivity AE[a]	5 (33.3%)	3 (30.0%)	8 (32.0%)
Any IAR	14 (93.3%)	10 (100.0%)	24 (96.0%)
Infusions interrupted due to AEs requiring medical intervention	7/387 (1.81%)	1/264 (0.38%)	8/651 (1.23%)

IAR: infusion-associated reaction; SAE, serious adverse event.
[a]Two subjects met anaphylaxis Standardised MedDRA Query but did not meet Sampson's criteria – events occurred day after infusion

Primary Endpoint: Safety

Dosing compliance was high: 96.1% and 99.2% in the 2.0 and 4.0 mg/kg/week groups, respectively. Of the 405 planned infusions in the 2.0 mg/kg/week treatment group, 18 (4.4%) were missed, and of the 270 planned infusions in the 4.0 mg/kg/week treatment group, six (2.2%) were missed.

Over the 27-week primary treatment phase, administration of elosulfase alfa was generally safe and well tolerated at both doses. No new or unexpected safety signals as compared to the other studies were observed. No patients reported AEs that led to permanent discontinuation of elosulfase alfa or study procedures. There was one serious AE (SAE; Table II), i.e., a hospitalization for a medical device (lower extremity metal plates) removal in a patient in the 4.0 mg/kg/week treatment group. The event was judged unrelated to the study drug and was rated moderate in severity. The most common AEs reported by the investigator as study drug-related were headache (46.7%), pyrexia (40.0%), vomiting (33.3%), nausea (33.3%), abdominal pain (20.0%), and fatigue (20.0%) in the 2.0 mg/kg/week treatment group and headache, nausea, abdominal pain, fatigue, cough, and dizziness in the 4.0 mg/kg/week treatment group (all reported by 20% of patients). All drug-related AEs were mild to moderate in severity. The number of infusions that were interrupted due to AEs and required medical intervention was low: 7/387 (1.8%) in the 2.0 mg/kg/week group and 1/24 (0.4%) in the 4.0 mg/kg/week group (Table II). No infusions in either treatment group had to be discontinued. All patients received and tolerated subsequent infusions.

No clinically meaningful changes in vital signs, clinical chemistry, hematology, or urinalysis results were observed. None of the patients had a shift to a clinically significant abnormal ECG from baseline to week 24.

All patients tested positive for anti-elosulfase alfa TAb by week 6 and remained positive for the duration of the study. Mean TAb titers were similar across dose groups at each study visit. All patients tested positive for NAb at least once during the study and most (23/25) remained positive afterwards. No patients tested positive for elosulfase alfa IgE during the study. No association was found between TAb or NAb positivity and decreases in normalized uKS. Nor was an association found between drug exposure and occurrence of treatment-emergent AEs toxicity grade $\geq$2, hypersensitivity AEs, or TAb or NAb titers. As the overall number of subjects who experienced hypersensitivity AEs was low (eight patients), it was difficult to reliably identify a relationship between TAb titers and hypersensitivity AEs.

TABLE III. Change From Baseline to Week 24 in 6-min Walk Test (6MWT), 3-min Stair Climb Test (3MSCT), and Urine Keratan Sulfate (uKS). Modified Intention-To-Treat Analysis Set in Elosulfase Alfa 2.0 and 4.0 mg/kg/week Dose Groups and in the Whole Study Group (Total)

	2.0 mg/kg/week N = 15	4.0 mg/kg/week N = 10	Total N = 25
6MWT distance change from baseline, m			
Median	1.4	−1.2	1.4
IQR	−32.1, 14.5	−9.9, 5.1	−29.3, 6.9
3MSCT change from baseline, stairs/min[a]			
Median	−1.3	13.9	4.8
IQR	−5.2, 5.3	5.3, 25.8	−3.6, 11.3
uKS change from baseline, μg/mg[b]			
Median	−4.8	−8.4	−6.3
IQR	−11.1, −1.2	−14.9, -6.0	−14.7, -1.9

IQR: interquartile range.
[a]One patient in the 2.0 mg/kg/week group did not perform the 3MSCT at week 24.
[b]One patient in the 2.0 mg/kg/week group and one patient in the 4.0 mg/kg/week group had no uKS data at both baseline and week 24.

Secondary Endpoints

6MWT, 3MSCT, and uKS. Table III shows changes from baseline at week 24 in 6MWT, 3MSCT, and uKS for the 2.0 and 4.0 mg/kg/week dosing cohorts and for the whole patient group. At baseline, the mean distance walked in the 6MWT was 372.2m (SD 80.6), and patients climbed a mean of 65.0 stairs (SD 21.7) per min in the 3MSCT (Table I). No changes from baseline were seen in the 6MWT at either dose at 12 or 24 weeks (Table III). Numerical improvements from baseline in the 3MSCT were seen in the 4.0 mg/kg/week group, but not in the 2.0 mg/kg/week group (Table III). Baseline normalized uKS was 17.4 μg/mg normalized creatinine and decreased substantially in both treatment groups. Although there was a more rapid and larger mean percentage decline from baseline observed in the 4.0 mg/kg/week dose group (−37.4 and −55.5% in the 2.0 and 4.0 mg/kg/week groups, respectively, at week 24), mean absolute changes were similar between groups: −8.0 (SD 9.9) μg/mg and −10.7 (SD 6.1) μg/mg, respectively (Fig. 1; median changes and interquartile ranges are shown in Table III). Both groups achieved similar mean levels of uKS at week 24, i.e., 8.2 μg/mg (SD 6.1) and 7.4 μg/mg (SD 4.2) in the 2.0 and 4.0 mg/kg/week groups, respectively.

Pharmacokinetics. Pharmacokinetic parameters for both doses of elosulfase alfa are listed in Appendix 2 (see Supporting Information online). Half-life ($t_{1/2}$) was similar in both dosing groups. The mean $t_{1/2}$ was approximately 6 min at week 0 for both dosing groups and increased to 23.2 and 31.1 min at week 23 for the 2.0 and 4.0 mg/kg/week dose groups, respectively. Following repeat dosing, $AUC_{0\text{-}t}$ and C_{max} increased by 48 and 44%, respectively, at week 23 compared to week 0 for the 2.0 mg/kg/week dose group, and by 69 and 100%, respectively, for the 4.0 mg/kg/week dose group. Differences between the 2.0 and 4.0 mg/kg/week doses in the mean $AUC_{0\text{-}t}$ and C_{max} were greater than dose proportionately, which indicates that the pharmacokinetics of elosulfase alfa are not linear over this dose range. Evaluation of pharmacokinetic versus pharmacodynamics and efficacy data showed a positive relation between elosulfase alfa exposure ($AUC_{0\text{-}t}$ and C_{max}) at week 23 and uKS % change from week 0 to week 24. A positive correlation was also found with changes in the 3MSCT, but not with changes in the 6MWT or maximum voluntary ventilation (MVV). The correlation with 6MWT, 3MSCT, and MVV was assessed as these were important efficacy measures in the phase 3 study. These results should be interpreted with caution because of the limited number of patients in these analyses.

Other secondary endpoints. Because of the small sample size and because differences between the 2.0 and 4.0 mg/kg/week groups in the endurance endpoints were difficult to interpret due to the heterogeneity of the disease, data from both dosing groups are presented together for the remainder of the secondary efficacy endpoints (Table IV and Appendix 3 in Supporting Information online).

CPET was conducted in 10 patients receiving elosulfase 2.0 mg/kg/week and in five patients receiving 4.0 mg/kg/week. At baseline, weight-adjusted peak oxygen uptake (VO_2) was mildly/moderately impaired (Appendix 3 in supporting information online), with a mean baseline value of 30.7 ml/kg/min (SD 7.5) (62% of predicted on average). There was no evidence of dynamic cardiac impairment in these subjects based on relatively stable ejection fraction measurements. CPET data at 25 weeks showed a positive change in exercise capacity: exercise duration, peak workload, and O_2 pulse increased with treatment (Table IV, Fig. 2, Appendix 3 in Supporting Information online). Oxygen uptake relative to work (VO_2/watt) decreased (improved) (Table IV), indicating that patients were performing work at a reduced oxygen cost.

Overall, pulmonary function tests showed positive changes from baseline at week 24 for most test variables (Table IV), consistent with the phase 3 study.

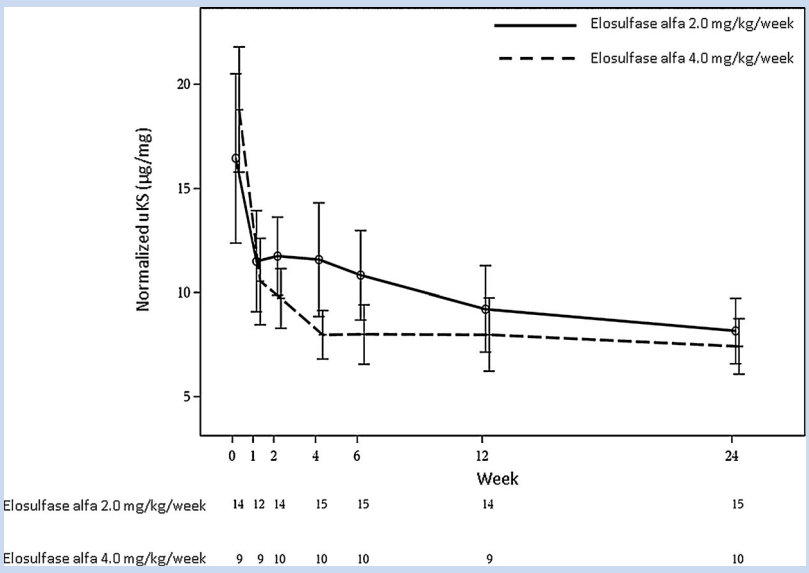

FIG. 1. Mean normalized urine keratan sulfate (uKS) versus time. Modified intention-to-treat population. Error bars represent standard errors.

TABLE IV. Median % Change From Baseline to Week 24/25 in Cardio-Pulmonary Function Test (CPET), Respiratory Function Tests, and Muscle Strength Tests and Pain Evaluation (Adolescent Pediatric Pain Tool, APPT) in the Whole Study Group. Modified Intention-To-Treat Analysis Set

	N	Median % change (IQR)
CPET		
Exercise duration, min	15	+16.9 (1.0, 23.1)
Peak VO_2, ml/kg/min	15	+5.3 (−6.3, 31.7)
O_2 pulse, ml/beat	15	+10.7 (1.3, 19.8)
Peak workload, watts	15	+26.5 (5.1, 42.4)
Aerobic efficiency, ml/watt	14	−7.6 (−11.9, 0.8)
Respiratory function		
Maximum voluntary ventilation, L/min	21	+6.2 (−7.7, 21.2)
Forced vital capacity, L	24	+5.4 (0.6, 10.4)
Forced expiratory volume in 1 s, L	24	+3.4 (−4.7, 12.2)
Forced inspiratory vital capacity, L	24	−0.4 (−5.3, 9.3)
Forced expiratory time, s	21	+2.0 (−4.1, 36.5)
Total lung capacity, L	16	+0.3 (−7.8, 17.1)
Muscle strength		
Knee extension, Nm	25	+11.5 (−9.4, 26.5)
Knee flexion, Nm	24	+2.9 (−20.2, 19.3)
Elbow flexion, Nm	19	+7.1 (−28.1, 43.6)
APPT		
Pain intensity	19	−30.6 (−74.4, 4.1)

IQR: interquartile range.

Baseline muscle strength tests suggested a population with moderately impaired knee extension (Appendix 3 in Supporting Information online). The most meaningful numerical improvements in muscle strength versus baseline at week 25 were seen in knee extension (a median improvement of 11%; Table IV); smaller improvements occurred in elbow and knee flexion.

The APPT results showed a mean baseline pain intensity score of 4.6 on the WGRS (Appendix 3 in Supporting Information online), corresponding to categorical scores of "medium pain" on the scale. The lower extremities were identified most frequently as an area where pain was experienced at baseline (by 68.0% of patients), followed by the upper extremities (32%) and the head and neck (32%). The mean pain intensity score on the APPT WGRS decreased (improved) numerically from 4.6 at baseline to 3.2 at 24 weeks (Appendix 3 in Supporting Information online), with the median % change from baseline being −30.6 (Table IV).

Echocardiogram data showed no clear evidence of an effect of elosulfase alfa on cardiac function after 24 weeks of therapy (data not shown). Home sleep testing showed no clear trends after 24 weeks (data not shown).

DISCUSSION

This study provides additional safety and tolerability data on the approved dose of elosulfase alfa (2.0 mg/kg/week) as well as a higher dose (4.0 mg/kg/week) in patients with Morquio A syndrome. In addition, this is the first study to comprehensively explore aspects of exercise capacity, muscle strength, and pain in Morquio A patients in order to provide new insights into the physiology and symptomatology of this disease.

The safety analysis did not reveal any new or unexpected safety signals, including IARs and hypersensitivity AEs, that were not observed in previous studies [Hendriksz et al., 2012; Hendriksz et al., 2014a]. Safety results were similar for the two dosing groups, with AEs reported by the investigator as study drug-related occurring in similar system organ classes and of similar type. In line with what has been described previously [Hendriksz et al., 2012; Hendriksz et al., 2014a], study drug-related AEs were mild to moderate in severity. The only SAE that was reported, hospitalization for medical device removal, was unrelated to the study drug. All patients developed anti-elosulfase alfa TAbs and NAbs during the study, which mostly remained positive for the duration of the study, but no relation between antibody titers and endurance outcomes, uKS changes, or hypersensitivity reactions could be established.

In the past, several studies have used endurance tests such as the 6MWT, and more recently the 3MSCT, to assess the impact of ERT on overall disease progression in MPS disorders [Harmatz et al., 2006; Hendriksz et al., 2014a; Muenzer et al., 2006; Wraith et al., 2004]. However, the physiological correlates of these tests are not well characterized. As indicated by the American Thoracic Society, the 6MWT evaluates the global and integrated responses of various systems working together, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism [American Thoracic Society, 2002]. In patients with Morquio A syndrome, multiple variables may contribute to performance in endurance tests, including changes in cardiovascular function, pulmonary function, skeletal muscle function, joint pain, and mechanical changes resulting from effects on skeletal dysplasia and deformity. This means that the impact of treatment on the 6MWT will differ between patients with different clinical manifestations. Despite limitations inherent to the study design, the present study collected important pilot data from a wide range of functional tests that may help to generate hypotheses regarding their interrelation with patient disabilities and how they are addressed with ERT.

The secondary efficacy measures 6MWT, 3MSCT, and uKS of the present study were the primary and secondary outcomes of the pivotal phase 3 study. The previous study showed differences between the placebo and elosulfase alfa 2.0 mg/kg/week groups after 24 weeks of treatment for 6MWT and uKS, but not for 3MSCT [Hendriksz et al., 2014a]. In the present uncontrolled study, uKS changed considerably from baseline after treatment in both dose groups, reaching similar mean and median absolute levels in both groups. No meaningful changes from baseline in 6MWT distance were seen in either dose group. The lack of impact in the 6MWT may be due to the inclusion criteria designed to recruit a study population healthy enough to complete the CPET, muscle strength tests, and other efficacy measures. Mean baseline walking distance was 372 m, considerably more than the values of around 200 m seen in the phase 3 study [Hendriksz et al., 2014a]. The fact that walking distances were closer to normal in this patient group (i.e., 70–80%

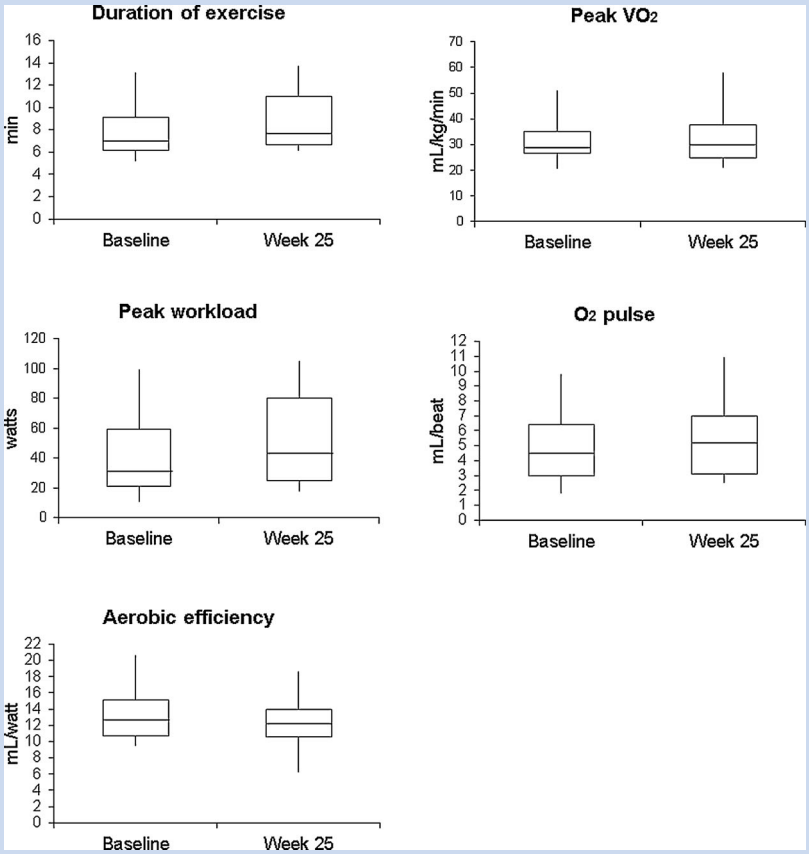

FIG. 2. Box plots showing median, interquartile ranges, and ranges of cardio-pulmonary exercise testing outcomes at baseline and week 25 (N = 15). Modified intention-to-treat population.

of that of an unaffected population [Lammers et al., 2008; Li et al., 2007]) may have made it more challenging to show improvement and could possibly account for the lack of change in 6MWT distance in this study. Also, these patients can probably not be expected to reach 6MWT distances comparable to healthy (unaffected) age/height-matched controls due to the presence of skeletal and joint abnormalities. The 6MWT is a multi-systemic test, though improvement may not be demonstrated in this endpoint in each individual benefiting from treatment due to the heterogeneity of the disease and variable impact of ERT on outcomes. Results of the 3MSCT also showed that patients in this study had a relatively high level of function, with the number of stairs climbed per minute at baseline being more than twice that seen in the phase 3 and MorCAP studies [Harmatz et al., 2013; Hendriksz et al., 2014a]. After 24 weeks of treatment, the 3MSCT showed a numerical improvement at the higher dose, but not at the lower dose, though there was a high degree of variability at both baseline and outcomes among subjects for this test. However, the present descriptive study was not powered to show statistically significant differences between groups given the large number of endpoints, the small sample size, and the clinical heterogeneity of the study population. Also, no conclusions can be made regarding the impact of elosulfase alfa on endurance based on these data as there was no control group. Larger controlled studies would be needed to further investigate the observed differences between dose groups.

The uKS data from both dosing groups suggested no obvious correlation with efficacy. Although a more rapid and larger percentage decline in uKS from baseline was observed with the 4.0 mg/kg/week dose than with the lower dose, the relevance of this finding is difficult to assess: Absolute changes in uKS were similar in both treatment groups, and both groups achieved a similar uKS level after 24 weeks. Moreover, as previously indicated, the study was not powered to detect significant differences between doses. uKS has been suggested to be a biomarker of clinical severity in Morquio A syndrome [Harmatz et al., 2013], but no clear link between treatment-induced uKS changes and clinical efficacy could be established in clinical studies thus far.

The most noteworthy findings of this study are that patients overall showed impaired exercise capacity and muscle strength and considerable pain, predominantly in the lower extremities, at baseline, several components of which showed numerical improvement after 24–25 weeks of treatment in both dose groups. The improvements seen in the 4.0 mg/kg/week dose group were larger than those seen in the 2.0 mg/kg/week dose group, particularly for the CPET and muscle strength variables. However, as the small sample size does not allow for any conclusions to be drawn on dose,

data were not shown for the different doses separately. It is interesting that the improvements seen in peak attainable workload and other CPET variables (exercise duration, O_2 pulse, and aerobic efficiency) were not associated with 6MWT distances, which remained essentially unchanged over the same period of time in the same study population. The contrasting results may have resulted partly from the difference in the effort-dependence of the two tests. Whereas the 6MWT is fundamentally a volitional test, largely depending on self-motivational factors that vary widely among patients, CPET is specifically designed to measure maximal exercise capacity while controlling for motivational factors. In the 6MWT, orthopedic abnormalities, such as genu valgum and ankle laxity/varus, could also explain why no increase was seen, even though exercise capacity was improved. At the same time, the observed improvements in aerobic efficiency suggest a mechanism for symptom improvement independent of exercise capacity, i.e., subjects experiencing less fatigue for the same amount of work performed. In the phase 3 study, patients treated with elosulfase alfa reported less fatigue than patients on placebo (data on file, BioMarin). The results of the present study provide a possible mechanism of action for explaining this observation. The CPET outcomes must be interpreted with caution as there could be a training effect that cannot be addressed due to the lack of a placebo group.

The APPT showed a pain burden at baseline, predominantly in the lower extremities, which confirms the findings from a patient-reported outcomes study in Morquio A patients in which 64% of 36 children and 74% of 27 adults reported joint pain, most often in the lower extremities [Hendriksz et al., 2014b]. Pain was also considerably reduced from baseline by ERT in this pilot study. A recent study reported a minimal clinically important difference of −12.5% in pain intensity, as determined by a 0–10 point numerical rating scale, in 153 adolescents with chronic pain [Hirschfeld et al., 2014], which is less than the reduction seen in our study (median change of −30.6%). Therefore, acknowledging the limitations of this small study, these findings suggest that the effect seen on pain intensity may be clinically important. The impact of treatment on respiratory function tests was limited. However, the patients in this study showed relatively good respiratory function (MVV and forced vital capacity) at baseline compared with the phase 3 study, which means that there may have been less room for improvement [Hendriksz et al., 2014a].

Overall, we can conclude that in this population of patients with a relatively good endurance/functional capacity, elosulfase alfa at 4.0 mg/kg/week had an acceptable safety and tolerability profile, similar to the profile previously observed at lower doses in more impaired populations. There was little change in endurance and respiratory endpoints, which might be due to the relatively healthy population (causing a ceiling effect), clinical heterogeneity, and small sample size. However, positive changes were observed in exercise capacity, muscle strength, and pain in subjects from both dose groups. These endpoints warrant further exploration as they may provide new insights into the physiology and symptomatology of Morquio A syndrome. The results of this study should be interpreted in light of the exploratory nature of the study, the small sample size, and the lack of a control group.

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SUPPORTING INFORMATION

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