

Immunogenicity of Elosulfase Alfa, an Enzyme Replacement Therapy in Patients With Morquio A Syndrome: Results From MOR-004, a Phase III Trial

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ABSTRACT

Purpose: Morquio A syndrome (mucopolysaccharidosis IVA [MPS IVA]) is a lysosomal storage disorder caused by deficiency of the enzyme *N*-acetylgalactosamine-6-sulfatase, which is required to degrade the glycosaminoglycan keratan sulfate. Morquio A is associated with extensive morbidity and early mortality. Elosulfase alfa is an enzyme replacement therapy that provides a treatment option for patients with Morquio A. We examined the immunogenicity profile of elosulfase alfa, assessing any correlations between antidrug antibodies and the efficacy and safety outcomes in 176 patients with Morquio A from a 24-week international Phase III trial.

Methods: Patients were randomized to placebo ($n = 59$) or elosulfase alfa 2.0 mg/kg administered weekly ($n = 58$) or every other week ($n = 59$) as an ~4-hour infusion. Blood samples were routinely tested to determine drug-specific total antibody titer and neutralizing antibody (NAb) positivity. Drug-specific immunoglobulin E positivity was tested routinely and in response to severe hypersensitivity adverse events (AEs). Antidrug antibody positivity and titer were compared with efficacy and safety metrics to assess possible correlations.

Findings: The 176 patients in the trial were 54% female, with a mean age of 11.9 years. In all patients treated with elosulfase alfa antidrug antibodies developed, and in the majority, antibodies capable of interfering with cation-independent mannose-6-phosphate receptor binding in vitro (NAb) developed. Less than 10% of patients tested positive for drug-specific IgE during the study. Despite the high incidence of anti-elosulfase alfa antibodies, no correlations were detected between higher total antibody titers or NAb positivity and worsened 6-minute walk

test results, urine keratin sulfate levels, or hypersensitivity AEs. Drug-specific IgE positivity had no apparent association with the occurrence of anaphylaxis, other hypersensitivity AEs, and/or treatment withdrawal.

Implications: Despite the universal development of antidrug antibodies, elosulfase alfa treatment was both safe and well tolerated and immunogenicity was not associated with reduced treatment effect. ClinicalTrials.gov identifier: NCT01275066. (*Clin Ther.* (Clin Ther. 2014;■:■■■-■■■) © 2014 Published by Elsevier HS Journals, Inc.

Key words: antibody, efficacy, enzyme replacement therapy, immunogenicity, Morquio A, mucopolysaccharidosis IVA.

INTRODUCTION

Morquio A syndrome (mucopolysaccharidosis IVA; OMIM 253000) is an autosomal recessive lysosomal storage disease caused by a deficiency of the enzyme *N*-acetylgalactosamine-6-sulfatase (GALNS; EC 3.1.6.4), which is required for degradation of the glycosaminoglycans chondroitin-6-sulfate and keratan sulfate (KS). Morquio A syndrome is a rare disease with an incidence estimated to range from 1 in 76,000 to 1 in 640,000 live births in different populations.¹⁻³

Patients with Morquio A syndrome appear initially healthy at birth but clinical signs due to the accumulation of glycosaminoglycans in multiple cells, tissues, and organs develop.⁴ Although the progression of

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Morquio A syndrome and the severity of individual clinical manifestations can vary considerably among patients, generally the burden of disease is severe, and patients have profound unmet medical needs.^{5,6} Patients frequently show prominent skeletal and connective tissue abnormalities, most commonly dwarfism with short trunk and neck, spinal abnormalities, genu valgum, pectus carinatum, hip dysplasia, and joint hypermobility and instability due to ligamentous laxity and joint bone deformities.⁵ Reduced endurance due to pulmonary and cardiac manifestations is also common, affecting both quality of life and mortality; for patients with Morquio A syndrome, death often results from cardiorespiratory or neurologic complications.⁵ Patients with rapidly progressing disease rarely live past their second or third decade, and even patients with more slowly progressing disease rarely live past their sixth decade.⁵

Enzyme replacement therapy (ERT) by infusion of recombinant human GALNS (elosulfase alfa^{*}) is approved by the US Food and Drug Administration and the European Commission for treatment of patients with Morquio A syndrome. Elosulfase alfa is currently the only approved therapy for Morquio A syndrome. Elosulfase alfa ERT is expected to reduce accumulated KS, which causes the Morquio A clinical manifestations.⁷ However, ERTs have the potential to induce antidrug antibodies in treated patients.^{8,9} Immune responses to ERTs for other lysosomal storage disorders are highly variable in terms of the incidence of antidrug antibodies and the impact on safety and efficacy.^{8–10} Here we report the immunogenicity profile of elosulfase alfa in patients with Morquio A syndrome during a 24-week Phase III clinical study (MOR-004) and examine the relationship between immunogenicity and patient outcomes and safety.

METHODS

Study Design and Patients

MOR-004 was a 24-week, randomized, double-blind, placebo-controlled Phase III study of elosulfase alfa in patients with Morquio A syndrome, conducted at 33 study centers in 17 countries from February 2011 to August 2012. After screening, 176 eligible patients who completed baseline assessments were

randomized to placebo (59 patients), elosulfase alfa 2.0 mg/kg/week (QW; n = 58) or elosulfase alfa 2.0 mg/kg/every other week (QOW; n = 59), administered in an infusion of ~4 hours; 1 patient from the QW group discontinued the study due to voluntary withdrawal of consent. The primary safety and efficacy outcomes of the MOR-004 trial have been reported.¹¹ In brief, the primary trial endpoint was the distance traveled during a 6-minute walk test (6MWT), a test of patient endurance and ambulation that was performed in accordance with American Thoracic Society guidelines.¹² To assess elosulfase alfa immunogenicity, antibody positivity and titer were assessed throughout the trial. The study protocol was approved by an institutional review board, independent ethics committee, or research ethics board at each participating clinical site. Each participant or his or her legally authorized representative provided written informed consent before entering the study. Patients, investigators, and site personnel were all blinded to treatment assignments until completion of final analyses.

Study participants had a documented clinical diagnosis of Morquio A syndrome based on clinical signs and symptoms, together with either a documented decrease in GALNS enzyme activity as assayed in fibroblasts or leukocytes or a genetic test confirming the Morquio A diagnosis according to the laboratory processes and procedures.¹³ Other inclusion criteria included an average 6MWT distance of 30 to 325 m during screening, anticipated ability to comply with the treatment schedule, and willingness to use an acceptable method of contraception. Exclusion criteria included undergoing a hematopoietic stem cell transplantation and major surgery within 3 months of study entry.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1975 Declaration of Helsinki, as revised in 2000. Each participant or his or her legally authorized representative provided written informed consent before entering the study in compliance with applicable local regulations.

Administration of Elosulfase Alfa

Patients received premedication with an antihistamine ~30 to 60 minutes before the start of the elosulfase alfa infusion. At the discretion of the investigator, patients with risk factors for

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infusion-associated reactions could receive additional agents before infusion, such as a sedating antihistamine (eg, diphenhydramine or chlorpheniramine), H₂ blockers, montelukast sodium, and corticosteroids. Elosulfase alfa 2.0 mg/kg or placebo solution was diluted to a final volume of either 100 or 250 mL with saline solution, allowed to reach room temperature, and administered intravenously. Patients randomized to the QOW group received infusions of elosulfase alfa or placebo on alternating weeks. Infusions were administered over ~4 hours.

Efficacy and Safety Evaluations

Urine KS (uKS) and creatinine were measured by quantitative analysis of samples obtained from first morning voids on 2 separate days at baseline, at weeks 2 and 4, and every 4 weeks thereafter until week 24, as well as within 1 week of an early withdrawal. Concentrations of uKS were normalized to urine creatinine concentrations.

Safety was evaluated by examination of the incidence, severity, and relationship to the study drug of treatment-emergent adverse events (AEs) reported during the study.¹¹ Potential hypersensitivity AEs were identified using the broad Anaphylactic Reaction Algorithmic Standardized Medical Dictionary of Regulatory Activities query (SMQ), version 15.0; (<http://www.meddra.org/>) and the broad angioedema SMQ using specific preferred terms. The broad algorithmic anaphylactic search strategy combines a number of anaphylactic reaction symptoms to refine the identification of cases consistent with Sampson criteria.¹⁴

Immunogenicity Analysis

Serum samples for assessment of immunogenicity were collected before dose administration at baseline, weeks 2 and 4, and every 4 weeks thereafter until week 24, and within 1 week of an early withdrawal visit. Additional blood samples were drawn for immunoglobulin E (IgE) testing if a patient experienced a severe infusion-associated reaction or an infusion-associated reaction requiring infusion cessation or at the investigator's discretion.

Titers of total elosulfase alfa-specific antibody (TAB) were determined by a validated bridging electrochemiluminescence assay that measures multiple antidrug antibody isotypes in a single assay. Anti-elosulfase alfa antibodies that bind and bridge biotin

and ruthenium-conjugated elosulfase alfa were captured by immobilized streptavidin and detected by electrochemiluminescence signal produced by ruthenium in response to an applied voltage. TAB-positive samples were confirmed for specificity against elosulfase alfa and serially diluted to obtain a titer. The TAB assay sensitivity is 1.59 ng/mL of positive control antibody.

The neutralizing antibody (NAb) assay used in this study detects elosulfase alfa-specific NAb capable of inhibiting elosulfase alfa from binding to cation-independent mannose-6-phosphate receptor (CI-M6PR) *in vitro*. Because elosulfase alfa is not enzymatically active within the neutral pH of blood, no assay was developed to detect antibodies that inhibit enzymatic activity. The presence of NAb, which is a subset of TAB, was assessed if a patient had a positive TAB result. The validated qualitative NAb assay was used to determine whether patient TAB could neutralize binding of biotin-labeled elosulfase alfa to CI-M6PR immobilized on an ELISA plate. Receptor-bound biotinylated elosulfase alfa was detected with streptavidin coupled to horseradish peroxidase. Samples screened as positive were confirmed for elosulfase alfa specificity, and the results were reported as positive or negative for NAb. The NAb assay sensitivity was 2.16 µg/mL of positive control antibody.

Anti-elosulfase alfa IgE was detected by a validated sandwich radioimmunoassay performed at ViraCor-IBT Laboratories (Lee's Summit, Missouri). In this qualitative assay, elosulfase alfa was covalently immobilized to filters, and drug-bound IgE was detected with radiolabeled anti-IgE antibodies. Elosulfase alfa specificity was confirmed for samples screened as positive for antidrug IgE. IgE assay results were reported as positive or negative for anti-drug IgE. Because a purified drug-specific positive control was not available, positivity was determined using a heterologous calibrator curve generated with rye grass-specific IgE. The sensitivity of the heterologous assay is ~0.242 ng/mL of anti-rye grass IgE.

Statistical Analyses

The TAB titer and proportions of patients with TAB-, NAb-, and IgE-positive results were summarized by treatment group at each scheduled visit. The relationships of immunogenicity and other end points, including safety, efficacy (6MWT), and pharmacodynamics (normalized uKS), were explored using summary

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statistics and graphic displays (boxplots and scatter-plots). Summaries and plots were produced across various patient classification categories defined as (1) TAB titer quartile based on the mean of postbaseline

TAB measurements, (2) TAB titer low/high groups defined by comparing each patient's mean postbaseline TAB titer with the overall mean, (3) positivity of baseline TAB defined as greater than or equal to the

Table 1. Incidence of anti-elosulfase alfa antibody positivity by visit.

Study Visit	Placebo (n = 59)	Elosulfase Alfa 2.0 mg/kg QOW (n = 59)	Elosulfase Alfa 2.0 mg/kg QW (n = 58 [*])
TAB titer positive patients, [†] no./study visit total [‡] (%)			
Baseline	12/59 (20.3)	15/59 (25.4)	9/58 (15.5)
Week 2	7/59 (11.9)	18/58 (31.0)	36/56 (64.3)
Week 4	13/58 (22.4)	43/59 (72.9)	56/56 (100.0)
Week 8	11/58 (19.0)	58/59 (98.3)	55/55 (100.0)
Week 12	14/59 (23.7)	57/58 (98.3)	56/56 (100.0)
Week 16	10/59 (16.9)	58/58 (100.0)	56/56 (100.0)
Week 20	16/59 (27.1)	59/59 (100.0)	57/57 (100.0)
Week 24	8/59 (13.6)	59/59 (100.0)	54/55 (98.2)
Overall	35/59 (59.3)	59/59 (100.0)	57/58 ^a (98.3)
NAb positive, no./study visit total [‡] (%)			
Baseline	0/59	0/59	0/58
Week 2	0/59	4/58 (6.9)	7/56 (12.5)
Week 4	0/58	12/59 (20.3)	43/56 (76.8)
Week 8	0/58	42/59 (71.2)	50/55 (90.9)
Week 12	3/59 (5.1)	48/58 (82.8)	49/56 (87.5)
Week 16	2/59 (3.4)	49/58 (84.5)	54/56 (96.4)
Week 20	1/59 (1.7)	51/59 (86.4)	53/57 (93.0)
Week 24	2/59 (3.4)	47/59 (79.7)	48/55 (87.3)
Overall	4/59 (6.8)	57/59 (96.6)	57/58 ^a (98.3)
IgE positive, no./study visit total [‡] (%)			
Baseline	1/59 (1.7)	0/59	2/58 (3.4)
Week 2	0/59	1/59 (1.7)	0/55
Week 4	0/58	1/58 (1.7)	0/56
Week 8	0/58	1/59 (1.7)	1/55 (1.8)
Week 12	0/59	0/58	1/57 (1.8)
Week 16	0/59	1/58 (1.7)	0/56
Week 20	0/59	0/59	0/57
Week 24	0/58	0/58	2/55 (3.6)
Overall	1/59 (1.7)	4/59 (6.8)	5/58 ^a (8.6)

IgE = immunoglobulin E; Nab = neutralizing antibody; QOW = every other week; QW = weekly; Tab = total antibody.

^{*}One patient in the QW cohort withdrew before study completion due to voluntary withdrawal of consent but is included in the overall listing of cumulative antibody positivity. The patient withdrew at week 2 without TAB, NAb, or IgE antibody positivity at any visit.

[†]TAB titer result equal to or greater than minimum required dilution (1:10).

[‡]Totals for each study visit are the number of samples collected for immunogenicity analysis.

minimum required dilution (1:10), (4) treatment-emergent hypersensitivity AE severity, and (5) the presence of treatment-emergent anaphylaxis or hypersensitivity AE.

RESULTS

MOR-004 was a randomized, double-blind, multinational Phase III trial designed to evaluate the efficacy and safety of elosulfase alfa in patients with Morquio A syndrome treated over 24 weeks.¹¹ Patients with a documented clinical diagnosis of Morquio A¹³ received placebo, elosulfase alfa 2.0 mg/kg QW, or elosulfase alfa 2.0 mg/kg QOW as an ~4-hour infusion. The primary safety and efficacy results have been reported.¹¹ In brief, the primary end point was change in the 6MWT from baseline to week 24; elosulfase alfa 2.0 mg/kg QW showed a statistically significant improvement in 6MWT compared with placebo, but elosulfase alfa 2.0 mg/kg QOW did not. This study examined the immunogenicity of elosulfase alfa and evaluated the effects of antibody formation on the overall efficacy and safety of elosulfase alfa in patients with Morquio A syndrome.

Patient Characteristics

The demographics and baseline characteristics of patients in the MOR-004 study have been published.^{11,15} In brief, patient ages ranged from 5 to

57 years, with a median age of 11.9 years; 52.3% were female. The treatment groups were balanced in demographic characteristics and baseline disease assessments.

Immunogenicity

In all patients treated with elosulfase alfa, anti-elosulfase alfa antibodies developed ([Table I](#)). The assay used to detect these antibodies is a bridging ELISA, capable of measuring all antidrug antibody isotypes present in the patient sample. These antibody results are referred to as TAB throughout this article. Patients in the QW cohort tested positive for TAB at a faster rate than patients in the QOW cohort ([Table I](#)); all QW patients were positive for TAB by week 4, and all QOW patients were positive for TAB by week 16. The mean TAB titers initially increased with time for both dosing cohorts, and then levels were maintained between weeks 16 and 24 ([Supplemental Table I, Figure 1](#)). The mean TAB titers by week 24 were similar in both dosing cohorts ([Table I, Supplemental Table I, Figure 1](#)). Approximately 20% of all patients (36 of 176) tested positive for TAB against elosulfase alfa at baseline ([Table I](#)). Both baseline-positive and baseline-negative patients who received elosulfase alfa had similar TAB titers at week 24 (data not shown).

NAb capable of inhibiting the drug from binding to the CI-M6PR in vitro were assessed in this study.

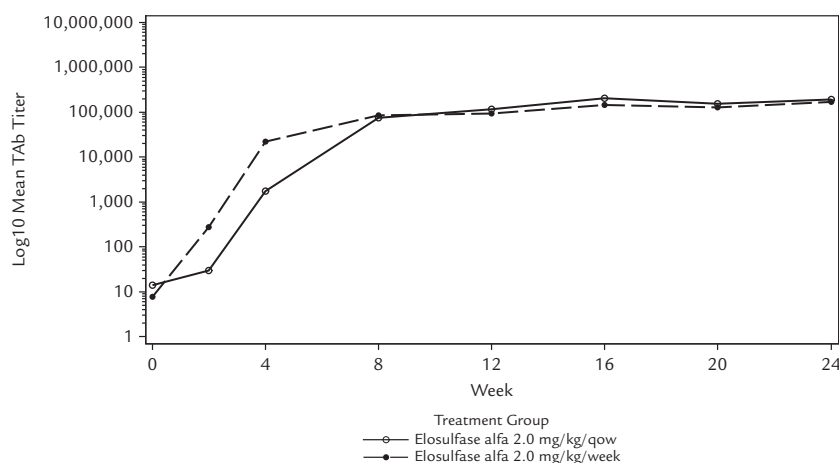


Figure 1. Mean total antidrug antibody (TAB) titers over 24 weeks for patients receiving elosulfase alfa. Mean TAB titers for the weekly and every other week elosulfase alfa dosing cohorts are presented by study week.

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CI-M6PR binding is necessary for elosulfase alfa uptake into lysosomes, the site of pharmacological activity.⁷ Antibodies capable of neutralizing enzymatic activity were not measured because elosulfase alfa is not active at the neutral pH of blood, but rather is active within the acidic environment of the lysosome, where antibody binding is not a concern. The majority of patients tested positive for NAb during the study (Table I). Eighty-seven percent of patients (48 of 55)

from the QW cohort tested positive for NAb at the week 24 study visit, and 80% (47 of 59) from the QOW cohort tested positive for NAb at the week 24 study visit. NAb positivity was slightly higher in the QW cohort than in the QOW cohort at each study week assessed.

Throughout the 24-week trial, <10% of patients in either dosing cohort tested positive for elosulfase alfa-specific IgE, with 8.6% of patients (5 of 58) from

Table II. Immunogenicity and hypersensitivity adverse events in IgE-negative patients.

AE Severity	Elosulfase Alfa 2.0 mg/kg QOW (n = 59)	Elosulfase Alfa 2.0 mg/kg QW (n = 58)
No. of IgE-negative patients	55	53
Patient incidence, no. (%)		
None	40 (72.73)	43 (81.13)
Mild	10 (18.18)	6 (11.32)
Moderate	4 (7.27)	3 (5.66)
Severe	1 (1.82)	1 (1.89)
No. of events/mean per patient		
Mild	17/0.31	10/0.19
Moderate	5/0.09	5/0.09
Severe	1/0.02	1/0.02
Mean TAB titer, ng/mL		
None	112,148	90,008
Mild	69,281	78,288
Moderate	64,533	249,833
Severe	65,641	35,426
Mean NAb rate positivity, %*		
None	64	75
Mild	58	88
Moderate	61	90
Severe	71	86
Mean NAb rate positivity, %*		
None	64	75
Mild	58	88
Moderate	61	90
Severe	71	86

AE =, adverse event; IgE = immunoglobulin E; Nab = neutralizing antibody; QOW = every other week; QW = weekly; Tab = total antibody.

Hypersensitivity adverse events were identified with the broad Anaphylactic Reaction Algorithmic Standardized Medical Dictionary of Regulatory Activities (MedDRA) query and the broad Angioedema Standardized MedDRA query (version 15.0). Patients with multiple AEs are assigned the highest severity.

*Based on postbaseline assessments; negative screen and titers were assigned a value of 0, and those less than the minimum required dilution were assigned a value of 5.

the QW cohort and 6.8% of patients (4 of 59) from the QOW cohort (Table I). With 1 exception, patients who tested positive had antidrug IgE detected at only a single study visit (data not shown).

Immunogenicity and Safety

Potential hypersensitivity AEs were identified using broad anaphylactic reaction SMQ and angioedema SMQ (Medical Dictionary of Regulatory Activities 15.0) and were examined for associations with immunogenicity. When the possible effects of TAB titer on hypersensitivity AEs were examined, antidrug IgE-positive patients were removed from analysis under the assumption that IgE positivity would be related to the occurrence of hypersensitivity events. When patients in both dosing cohorts were divided by the presence or absence of either anaphylaxis or hypersensitivity AEs, the mean TAB titers were similar in both groups (Supplemental Figure 1). In both dosing cohorts, when patients were analyzed by their most severe hypersensitivity AE, there was no consistent correlation between maximum severity and mean TAB titer (Table II). Additionally, there did not appear to be any clear correlations between NAb positivity and

hypersensitivity AEs: for patients in both dosing cohorts, the percentage of visits at which a patient was NAb positive did not correlate with the severity of hypersensitivity AEs (Table II). The presence or absence of TAB positivity at baseline did not correlate with the incidence or maximum severity of hypersensitivity AEs (Supplemental Table II).

Most patients with serious AEs (9 of 58 QW patients and 4 of 59 QOW patients¹¹), including 3 patients with drug-related serious AEs of hypersensitivity, vomiting, and anaphylactic reaction, did not test positive for antidrug IgE at any time during the study, and patients positive for antidrug IgE did not have any reported severe hypersensitivity AEs (data not shown).

Immunogenicity and Pharmacodynamics and Efficacy

For patients in both dosing cohorts, a similar percentage of change in uKS levels between baseline and week 24 were observed regardless of TAB titer quartile (Figure 2, Supplemental Figure 2). Additionally, no association was observed between the percentage of visits at which a patient was NAb positive and the

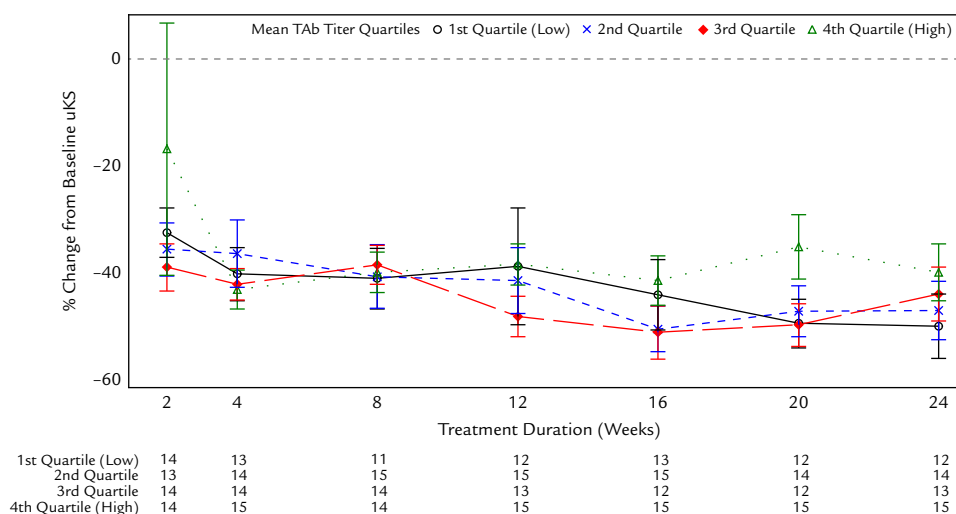


Figure 2. Changes in urine keratan sulfate (uKS) levels over 24 weeks for patients receiving weekly doses of elosulfase alfa, by total antidrug antibody (TAB) quartile. Patients in the weekly dosing cohort were divided by mean TAB titer quartile over 24 weeks, with the first quartile representing patients with the lowest titers and the fourth quartile representing patients with the highest titers. For each TAB titer quartile, the mean percentage of change in creatinine-normalized uKS levels from baseline is presented by study week. Error bars show SE.

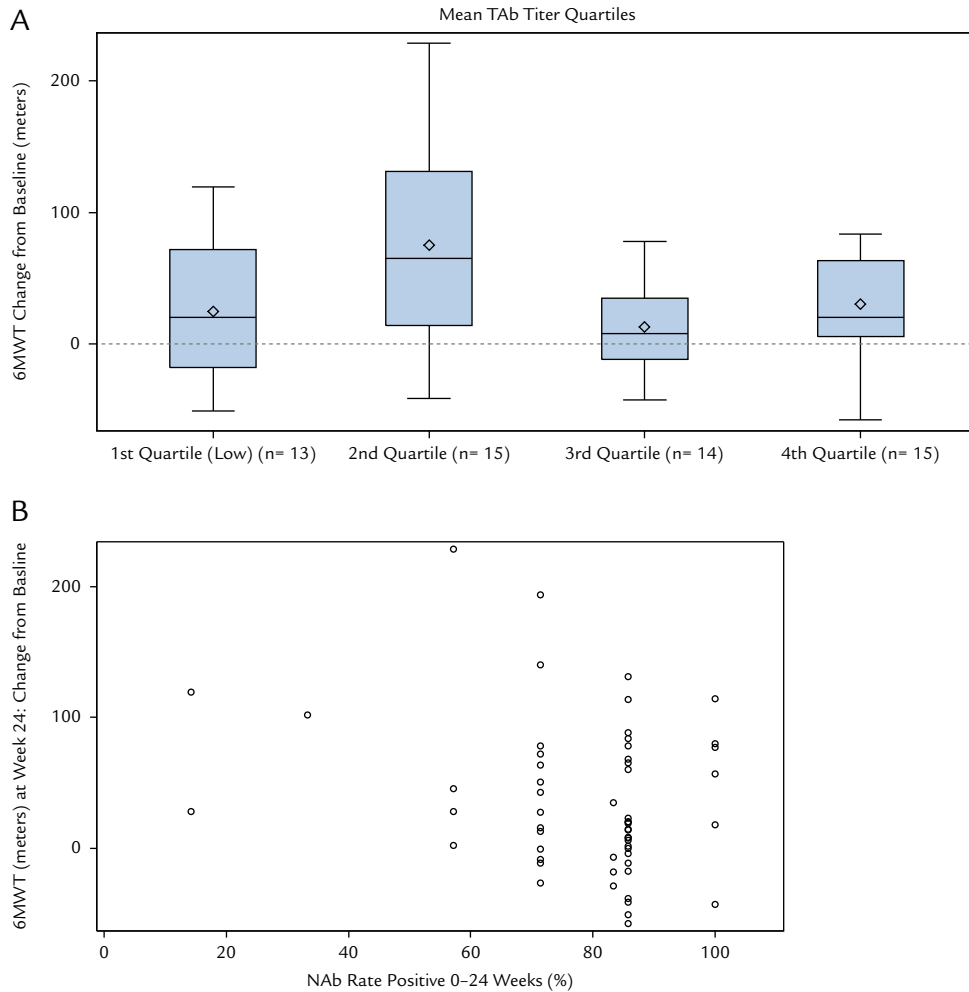


Figure 3. Efficacy and immunogenicity for patients receiving weekly elosulfase alfa. **A**, Changes in 6-minute walk test (6MWT) distance from baseline to week 24, by total antidrug antibody (TAB) titer quartile. Patients in the weekly dosing cohort were divided by mean TAB titer quartile over 24 weeks, with the first quartile representing patients with the lowest titers and the fourth quartile representing patients with the highest titers. For each TAB titer quartile, patient changes in 6MWT distances from baseline to week 24 are presented as boxplots. **(B)** Changes in 6MWT distance from baseline to week 24, by neutralizing antidrug antibody (NAb) positivity rate. For patients in the weekly dosing cohort, change in the 6MWT between baseline and week 24 is plotted against the percentage of study visits for which a patient was positive for NAb.

percentage of change in uKS levels between baseline and week 24 ([Supplemental Figure 3](#)).

No associations were evident between TAB titers or NAb positivity and patient efficacy outcomes in either elosulfase alfa dosing cohort, as measured by the 6MWT ([Figure 3](#)). Patients with higher TAB titers ([Figure 3A](#)) or higher NAb positivity rates ([Figure 3B](#)) in the QW cohort did not have reduced 6MWT results

compared with patients with lower antibody responses. Similar results were observed for the QOW cohort ([Supplemental Figure 4](#)).

DISCUSSION

Anti-drug antibodies developed in all patients treated with elosulfase alfa. The universality of antidrug antibody development suggests that measurements of

elosulfase alfa antibody positivity or titer are unlikely to be useful predictors of which patients are at greater risk of clinical consequences related to immunogenicity. The relatively high incidence of antidrug antibody positivity at baseline and in the placebo cohort (~20%, [Table I](#)) is likely related to the conservative cut point of the assay, designed to reduce the possibility of yielding false-negative results. TAB titers in the placebo group were low and transient compared with those in patients who received elosulfase alfa, suggesting that the actual prevalence of preexisting anti-elosulfase alfa antibodies in untreated patients may be lower than indicated in this study. The overall lack of correlation detected between anti-elosulfase alfa antibody development and pharmacodynamic and efficacy outcomes may be explained by the rapid uptake of elosulfase alfa from plasma into cells by the CI-M6PR.⁷ The half-life of elosulfase alfa in plasma was ~35 minutes after repeated administration.¹⁵ Rapid uptake from plasma may limit drug exposure to circulating antibodies, which may in turn reduce the formation of immune complexes and the overall negative effects of antidrug antibodies.

Among different ERTs, immunogenicity and its relationship with patient efficacy and safety outcomes appear to be quite variable. In infant patients with Pompe disease treated with the ERT alglucosidase alfa, high antidrug antibody titers correlate with attenuated therapeutic responses.¹⁰ However, similar to the results observed in this trial, ERTs for Fabry disease, mucopolysaccharidosis I, and mucopolysaccharidosis VI have shown sustained therapeutic response, despite the development of antidrug antibodies.^{16–18} The immunogenicity of elosulfase alfa will continue to be monitored in the extension study and in the postapproval setting, including evaluations for any impact on long-term safety or efficacy.

CONCLUSIONS

Antidrug antibodies developed in all patients treated with elosulfase alfa, and in the majority, NABs capable of interfering with CI-M6PR binding in vitro developed. However, there were no apparent associations between higher TAB titers or NAB positivity and worsened 6MWT results, uKS levels, or hypersensitivity AEs. Development of drug-specific IgE positivity had no apparent association with the occurrence of anaphylaxis, other hypersensitivity AEs, or treatment withdrawal. These data further support

the use of elosulfase alfa treatment for patients with Morquio A syndrome.

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CONFLICTS OF INTEREST

All authors are employees of BioMarin Pharmaceutical Inc and stock holder of BioMarin.

SUPPLEMENTAL MATERIALS

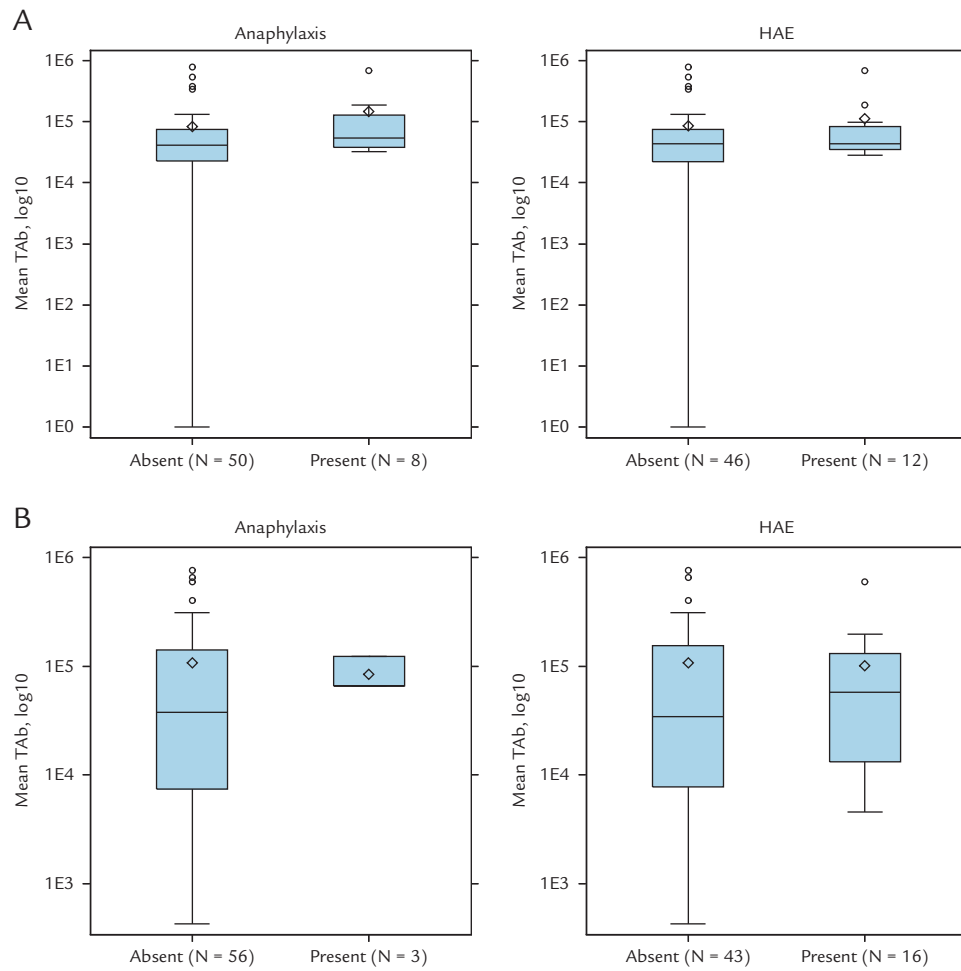
Supplementary material cited in this article is available online at <http://dx.doi.org/10.1016/j.clinthera.2014.11.005>.

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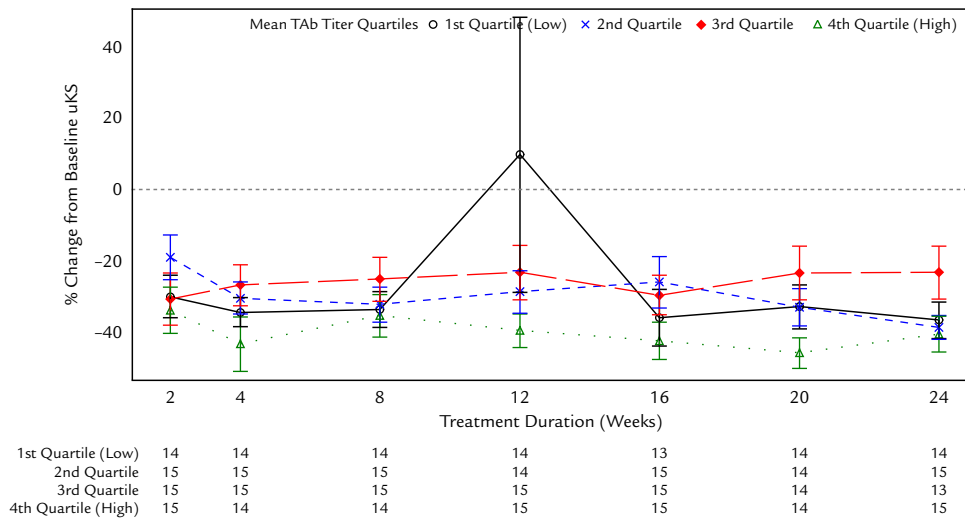
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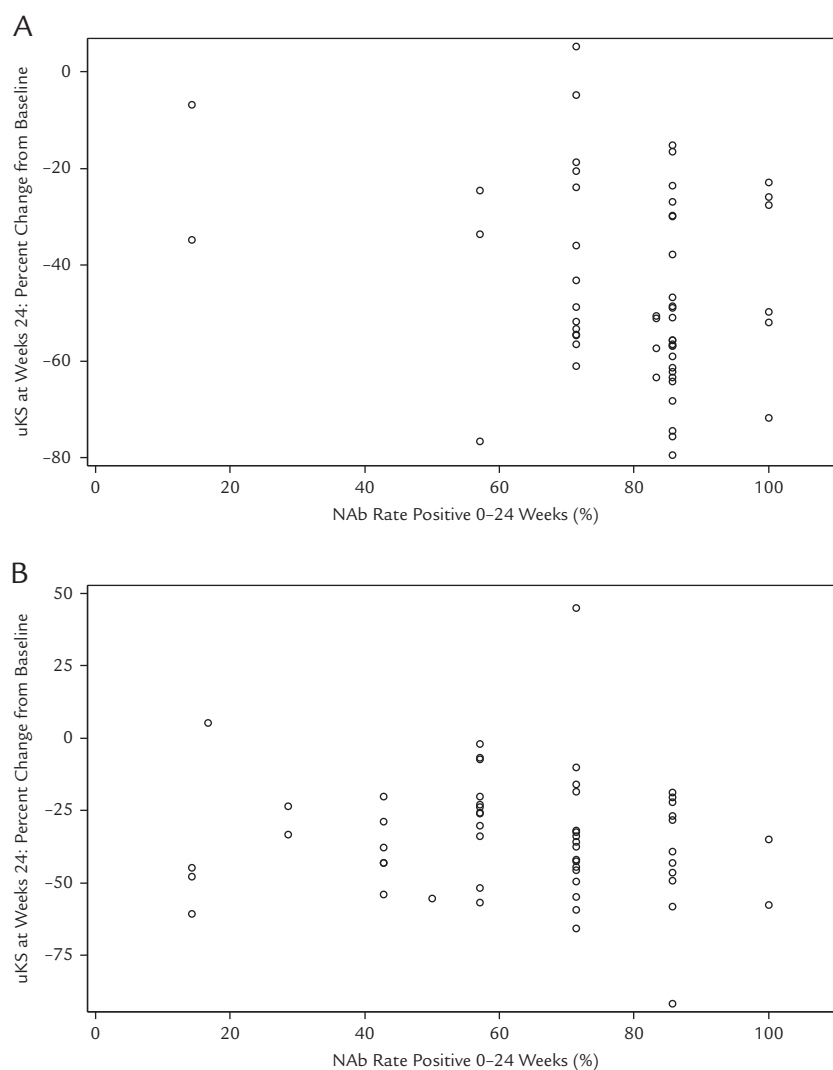
Address correspondence to: Becky Schweighardt, PhD, BioMarin Pharmaceutical Inc, 105 Digital Drive, Novato, CA 94949. E-mail: BSchweighardt@bmrn.com



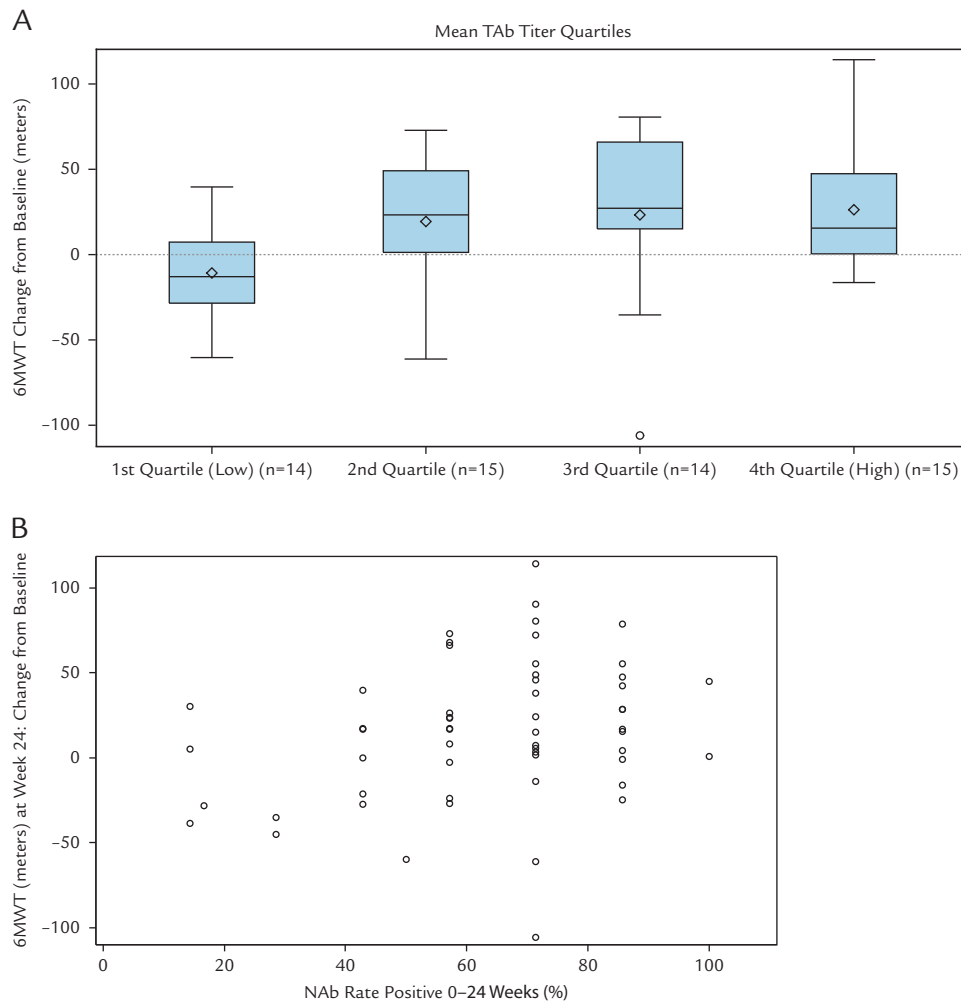
Supplemental Figure 1. Total antidrug antibody (TAb) titers in patients with and without anaphylaxis or hypersensitivity adverse event (HAE). Patients in the weekly dosing cohort (A) or every-other-week dosing cohort (B) were divided by the presence or absence of a reported anaphylaxis event (left) or an HAE (right). The mean TAb titers over 24 weeks for these patients are presented as boxplots.



Supplemental Figure 2. Changes in urine keratan sulfate (uKS) levels over 24 weeks for every-other-week dosing cohort by total antidrug antibody (TAB) quartile. Patients in the every-other-week dosing cohort were divided by mean TAB titer quartile over 24 weeks, with the first quartile representing patients with the lowest titers and the fourth quartile representing patients with the highest titers. For each TAB titer quartile, the mean percentage of change in creatinine-normalized uKS levels from baseline is presented by study week. Error bars show SE.



Supplemental Figure 3. Changes in urine keratan sulfate (uKS) levels over 24 weeks by neutralizing antidrug antibody (NAb) positivity percentage. For patients in the weekly dosing cohort (A) or the every-other-week dosing cohort (B), the percentage of change in creatinine-normalized uKS levels from baseline is plotted against the percentage of study visits for which that patient was positive for NAb.



Supplemental Figure 4. Efficacy and immunogenicity for the every-other-week (QOW) dosing cohort. **A**, Changes in the 6-minute walk test (6MWT) distance from baseline to week 24 by total antidrug antibody (TAB) quartile. Patients in the QOW dosing cohort were divided by mean TAB titer quartile over 24 weeks, with the first quartile representing patients with the lowest titers and the fourth quartile representing patients with the highest titers. For each TAB titer quartile, patient changes in 6MWT distances from baseline to week 24 are presented as boxplots. **B**, Changes in 6MWT distance from baseline to week 24, by neutralizing antidrug antibody (NAb) positivity. For patients in the QOW dosing cohort, the change in the 6MWT between baseline and week 24 is plotted against the percentage of study visits for which a patient was positive for NAb.

Supplemental Table I. Total anti-elosulfase alfa antibody titers by study visit week.

Study Visit	Placebo (n = 59)	Elosulfase alfa 2.0 mg/kg QOW (n = 59)	Elosulfase alfa 2.0 mg/kg QW (n = 58)
Baseline			
No.	59	59	58
Mean (SD)	4 (13.4)	14 (50.3)	8 (37.3)
Median	0	0	0
25th, 75th percentiles	0, 0	0, 5	0, 0
Minimum, maximum	0, 90	0, 270	0, 270
Week 2			
No.	59	58	56
Mean (SD)	3 (12.4)	29 (116.3)	272 (1026.1)
Median	0	0	30
25th, 75th percentiles	0, 0	0, 10	0, 90
Minimum, maximum	0, 90	0, 810	0, 7290
Week 4			
No.	58	59	56
Mean (SD)	4 (9.3)	1734 (5574.9)	22,093 (40,465.5)
Median	0	10	7290
25th, 75th percentiles	0, 0	0, 270	810, 21,900
Minimum, maximum	0, 30	0, 21,900	10, 197,000
Week 8			
No.	58	59	55
Mean (SD)	4 (13.4)	74145 (150,595.6)	83995 (137,951.9)
Median	0	21900	21900
25th, 75th percentiles	0, 0	810, 65,600	7290, 65,600
Minimum, maximum	0, 90	0, 590,000	2430, 590,000
Week 12			
No.	59	58	56
Mean (SD)	374 (2850.7)	117,229 (251,548.7)	94,734 (134,114.9)
Median	0	65,600	65,600
25th, 75th percentiles	0, 0	7290, 197,000	21,900, 65,600
Minimum, maximum	0, 21,900	0, 1,770,000	90, 590,000
Week 16			
No.	59	58	56
Mean (SD)	3 (7.7)	206,840 (402,339.4)	145,601 (265,404.6)
Median	0	65,600	65,600
25th, 75th percentiles	0, 0	7290, 197,000	21,900, 197,000
Minimum, maximum	0, 30	30, 1,770,000	2430, 1,770,000
Week 20			
No.	59	59	57
Mean (SD)	5 (14.4)	155,601 (189,210.9)	129,063 (264,828.4)
Median	0	65,600	65,600
25th, 75th percentiles	0, 5	21,900, 197,000	21,900, 65,600
Minimum, maximum	0, 90	810, 590,000	810, 1,770,000

(continued)

Clinical Therapeutics

Supplemental Table I. (continued).

Study Visit	Placebo (n = 59)	Elosulfase alfa 2.0 mg/kg QOW (n = 59)	Elosulfase alfa 2.0 mg/kg QW (n = 58)
Week 24			
No.	59	59	55
Mean (SD)	16 (105.8)	190,901 (343,722.0)	167,737 (398,716.8)
Median	0	65,600	65,600
25th, 75th percentiles	0, 0	21,900, 197,000	21,900, 65,600
Minimum, maximum	0, 810	810, 1,770,000	0, 1,770,000

QOW = every other week; QW = weekly.

Mean refers to the average of all titers from all patients at the specified study visit.

Supplemental Table II. Hypersensitivity AEs by baseline positivity for total anti-elosulfase alfa antibodies.

Total Antibody Titer Baseline Positive	Elosulfase Alfa 2.0 mg/kg QOW (n = 59)		Elosulfase Alfa 2.0 mg/kg QW (n = 58)	
	No (n = 44)	Yes (n = 15)	No (n = 49)	Yes (n = 9)
Patient incidence of hypersensitivity AEs, no. (%)				
None	31 (70.45)	12 (80.00)	38 (77.55)	8 (88.89)
Mild	8 (18.18)	3 (20.00)	7 (14.29)	0
Moderate	4 (9.09)	0	3 (6.12)	1 (11.11)
Severe	1 (2.27)	0	1 (2.04)	0
No. of events/mean per patient				
Mild	13/0.30	5/0.33	11/0.22	1/0.11
Moderate	5/0.11	0	3/0.06	3/0.33
Severe	1/0.02	0	1/0.02	0

AE = adverse event; QOW = every other week; QW = weekly.

Hypersensitivity adverse events were identified by utilizing the broad Anaphylactic Reaction algorithmic Standardized Medical Dictionary of Regulatory Activities (MedDRA) query and the broad Angioedema Standardized MedDRA query (version 15.0). Patients with multiple AEs are assigned the highest severity.