

# Long-Term Immunogenicity of Elosulfase Alfa in the Treatment of Morquio A Syndrome: Results From MOR-005, a Phase III Extension Study

Brian Long, PhD<sup>1</sup>; Troy Tompkins, BS<sup>1</sup>; Celeste Decker, MD<sup>1</sup>; Lynne Jesaitis, PhD<sup>1</sup>; Shahid Khan, MBBS<sup>1</sup>; Peter Slasor, ScD<sup>1</sup>; Paul Harmatz, MD<sup>2</sup>; Charles A. O'Neill, PhD<sup>1</sup>; and Becky Schweighardt, PhD<sup>1</sup>

<sup>1</sup>BioMarin Pharmaceutical Inc., Novato, California; and <sup>2</sup>Department of Gastroenterology, UCSF Benioff Children's Hospital Oakland, Children's Hospital and Research Center Oakland, Oakland, California

## ABSTRACT

**Purpose:** Elosulfase alfa is an enzyme replacement therapy for the treatment of Morquio A syndrome (mucopolysaccharidosis IVA), a lysosomal storage disorder caused by a deficiency of the enzyme N-acetylgalactose-amine-6-sulfatase. We previously reported immunogenicity data from our 24-week placebo-controlled Phase III study, MOR-004. Here, we report the long-term immunogenicity profile of elosulfase alfa from MOR-005, the Phase III extension trial to assess potential correlations between antidrug antibodies and efficacy and safety profile outcomes throughout 120 weeks of treatment.

**Methods:** The long-term immunogenicity of elosulfase alfa was evaluated in patients with Morquio A syndrome in an open-label extension study for a total of 120 weeks. All patients received 2.0 mg/kg elosulfase alfa either weekly or every other week before establishment of 2.0 mg/kg/wk as the recommended dose, at which time all patients received weekly treatment. Efficacy measures were compared with those from the MOR-004 baseline, enabling analysis of changes over 120 weeks. The primary efficacy measure was the change from baseline in 6-minute walk test. Secondary measures included changes from baseline in 3-minute stair climb test and normalized urine keratan sulfate, a pharmacodynamic metric.

**Findings:** All patients treated with elosulfase alfa developed antidrug total antibodies (TAb) by week 24 of MOR-004. In the extension study, all patients, including those who had previously received placebo, were TAb positive by study week 36 (MOR-005 week 12). All patients remained TAb positive throughout the study, and TAb titers were similar across treatment groups at week 120. Nearly all

patients tested positive for neutralizing antibodies (NAb) at least once, with incidence of NAb positivity peaking at 85.9% at study week 36, then steadily declining to 66.0% at study week 120. In all treatment groups, mean urine keratan sulfate remained below treatment-naïve baseline despite the presence of antidrug antibodies. No relationship was observed between TAb titers or NAb positivity and changes in urine keratan sulfate, 6-minute walk test, or 3-minute stair climb test from baseline to week 120. No consistent associations were detected between antidrug antibodies and the occurrence of hypersensitivity adverse events or anaphylaxis over the course of the study.

**Implications:** Immunogenicity results from this long-term study are consistent with previously reported 24-week results. Despite the sustained presence of antidrug antibodies, elosulfase alfa was well tolerated, and patients continued to benefit from treatment through week 120. No associations were detected between higher TAb titers or NAb positivity and reduced treatment effect or worsened safety profile measures. ClinicalTrials.gov identifier: NCT01415427. (*Clin Ther.* 2016;■:■■■-■■■) © 2016 The Authors. Published by Elsevier HS Journals, Inc.

**Key words:** elosulfase alfa, immunogenicity, Morquio A, mucopolysaccharidosis IVA, Vimizim.

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## INTRODUCTION

Morquio A syndrome (mucopolysaccharidosis IVA; OMIM 253000) is an autosomal recessive lysosomal storage disease that results from deficient activity of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase (GALNS; EC 3.1.6.4) and leads to the pathogenic accumulation of the glycosaminoglycans (GAGs) chondroitin-6-sulfate and keratan sulfate. Currently, >270 disease-associated mutations have been documented in the *GALNS* gene.<sup>1</sup>

The incidence of Morquio A syndrome is highly variable, depending on the population studied, with estimates ranging from 1 in 76,000 live births in Northern Ireland to 1 in 640,000 live births in Western Australia.<sup>2,3</sup> Children with Morquio A syndrome appear healthy at birth but decline progressively as GAGs accumulate in multiple tissues, resulting in multisystemic impairment.<sup>4,5</sup> Although symptom severity varies, patients frequently exhibit severe skeletal and connective tissue abnormalities, including short stature, spinal abnormalities, dysostosis multiplex, hip dysplasia, and joint instability.<sup>5–7</sup> Patients also exhibit pulmonary defects, contributing to a progressive decline in endurance.<sup>8</sup> Symptoms of Morquio A syndrome dramatically affect the quality of life and the life span of affected patients.<sup>9</sup> Although progression rates vary, patients with Morquio A syndrome rarely live past their sixth decade, and patients with more rapidly progressing disease may not live past their second or third decade. Death commonly results from cardiorespiratory or neurologic complications.<sup>5,10</sup>

Currently, the only therapy approved for the treatment of Morquio A syndrome is elosulfase alfa (Vimizim; BioMarin Pharmaceutical Inc, Novato, California), a recombinant form of human GALNS administered via infusion.<sup>11</sup> Elosulfase alfa contains mannose-6-phosphate-terminated oligosaccharide chains, which allow it to bind the cation-independent mannose-6-phosphate receptor (CI-M6PR) and be taken up into lysosomes. Once inside the lysosome, elosulfase alfa mediates the degradation and clearance of accumulated GAGs.<sup>12</sup> Enzyme replacement therapies (ERTs) have the potential to induce antidrug antibodies (ADAs) in treated patients, which may result in decreased efficacy over time.<sup>13–15</sup> Because Morquio A patients require treatment throughout their lives, it is vital that therapies retain effectiveness over a long treatment duration.

The efficacy, tolerability, and immunogenicity of elosulfase alfa have been explored in previous studies, including MOR-004, a double-blind, placebo-controlled Phase III study.<sup>16,17</sup> After 24 weeks of treatment with elosulfase alfa 2.0 mg/kg/wk, patients with Morquio A exhibited significant improvements in the 6-minute walk test (6MWT) and reduced levels of urine keratan sulfate (uKS) compared with placebo-treated patients. Elosulfase alfa was found to have an acceptable safety profile, with no adverse events (AEs) leading to permanent treatment discontinuation.<sup>16</sup> All patients treated with elosulfase alfa developed ADAs; however, no correlation was observed between higher antidrug total antibody (TA<sub>b</sub>) titer and reduced efficacy or worsened safety profile outcomes.<sup>17</sup>

## METHODS

### Study Design and Patients

MOR-005 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01415427) identifier: NCT01415427) was a long-term, open-label study designed to examine the tolerability and efficacy of elosulfase alfa in patients with Morquio A syndrome.<sup>18</sup> MOR-005 is an extension of MOR-004 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01275066) identifier: NCT01275066), a 24-week, double-blind, placebo-controlled Phase III clinical study.<sup>16</sup> All but 3 of the 176 patients completing the 24-week study entered the open-label portion. All participants had a confirmed diagnosis of Morquio A syndrome, based on signs and symptoms of disease as well as laboratory and/or genetic findings. Demographic and baseline characteristics of patients in MOR-005 have been previously reported.<sup>18</sup>

MOR-005 was divided into 2 parts, based on dosing regimens. Initially, patients randomized to receive elosulfase alfa in MOR-004 remained on their assigned dose of 2.0 mg/kg every week (QW-QW) or every other week (QOW-QOW). Patients who received placebo in MOR-004 were re-randomized to receive 1 of the 2 elosulfase alfa dose groups (PBO-QW, PBO-QOW). After establishment of the recommended dose, all patients were shifted to 2.0 mg/kg/wk for the remainder of the study. The specific study week of dose transition varied among patients, ranging from week 36 to week 96. MOR-005 was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and with the ethical principles of the Declaration of

Helsinki; all patients provided written informed consent before study enrollment.

### Pharmacodynamic, Efficacy, and Safety Profile Evaluations

uKS was measured by quantitative analysis of samples obtained from first morning voids. uKS concentrations were normalized to concentration of urine creatinine in the samples. In MOR-005, uKS was initially measured every 12 weeks and then shifted to every 24 weeks after dose establishment.

In MOR-005, the 6MWT and 3-minute stair climb test (3MSCT), both measures of endurance, were conducted twice on separate days every 12 weeks before dose establishment and every 48 weeks after dose establishment. Patients who were physically unable to perform the tests were scored as zero meters or stairs per minute, respectively. The 6MWT was performed according to the American Thoracic Society Guidelines.<sup>19</sup> The 3MSCT was performed as previously described.<sup>20</sup> Study site personnel were thoroughly trained and certified to minimize variability in the conduct of the 6MWT and 3MSCT.

Tolerability was assessed by determining the incidence and severity of hypersensitivity AEs (HAEs) and anaphylaxis reported during the study and assessing any potential correlation between HAEs and ADA positivity and/or titer. Potential HAEs were identified using the broad algorithmic anaphylactic reaction and broad angioedema Standardized Medical Dictionary of Regulatory Activities Queries (version 15.0; <http://www.meddra.org/>). Anaphylaxis cases were defined by a broad algorithmic anaphylactic search strategy to identify temporally related anaphylactic reaction symptoms consistent with the criteria published by the National Institute of Allergy and Infectious Disease and Food Allergy and Anaphylaxis Network in 2006.<sup>21</sup>

### Immunogenicity Assays and Sampling Frequency

Immunogenicity measures included determination of antidrug TAb titer, NAb positivity, and elosulfase alfa-specific immunoglobulin E (IgE) positivity. NAb was assessed only in patients with TAb positivity.

Serum samples for assessment of immunogenicity were collected before dose administration at every 12 weeks in MOR-005 before dose establishment and every 24 weeks after dose establishment. Additional blood samples were drawn for elosulfase alfa-specific

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.

TAbs were measured using a validated bridging electrochemiluminescence assay, which detects multiple isotypes of ADAs in one assay. Samples screened as positive for TAb were confirmed for specificity to elosulfase alfa and were serially diluted to obtain a titer. The TAb assay, which had a sensitivity of 1.59 ng/mL of positive control antibody, was performed as previously described.<sup>17</sup>

Because the CI-M6PR is required for the uptake of elosulfase alfa into lysosomes, NABs were defined as antibodies capable of inhibiting elosulfase alfa from binding to the CI-M6PR in vitro.<sup>12</sup> The NAb assay used in this publication specifically detects ADAs capable of inhibiting elosulfase alfa from binding to the CI-M6PR. The presence of antibodies that inhibit enzymatic activity of elosulfase alfa was not assessed in this study, because elosulfase alfa is active only within the acidic environment of the lysosome and inactive in the neutral pH of the blood. The NAb assay was a validated ligand-binding enzyme-linked immunosorbent assay that qualitatively assessed binding inhibition between biotin-labeled elosulfase alfa and immobilized CI-M6PR in vitro, as described previously.<sup>17</sup> Samples screened as positive for NAb were confirmed for elosulfase alfa specificity, and results were reported as positive or negative. The NAb assay sensitivity was 2.16 µg/mL of positive control antibody.

Anti-elosulfase alfa IgE was detected by a validated ligand binding radioimmunoassay performed at ViraCor-IBT Laboratories (Lee's Summit, Missouri). Because a purified anti-elosulfase alfa positive control was not available, IgE binding to immobilized elosulfase alfa was determined using a heterologous calibrator curve generated with rye grass-specific IgE. The sensitivity of the heterologous assay was ~0.242 ng/mL of anti-rye grass IgE. Additional details of this assay have been reported.<sup>17</sup>

### Statistical Analyses

The intention-to-treat (ITT) population includes all patients who were randomized to receive study treatment and received at least 1 dose of the study drug. The modified per-protocol (MPP) population excludes those patients who underwent orthopedic surgeries

(which were predicted to alter efficacy analyses) or who missed at least 20% of infusions. In this study, immunogenicity, tolerability, and pharmacodynamic variables were assessed in the ITT population ( $n = 173$ ), whereas effort-based efficacy variables (6MWT and 3MSCT) were assessed in the MPP population ( $n = 124$ ).<sup>18</sup>

Immunogenicity analyses (mean TAb titer and TAb, NAb, and IgE positivity) are reported per treatment group at select study weeks. The relationship of immunogenicity to tolerability, efficacy, and pharmacodynamic end points was explored using summary statistics and graphic displays (box plots and scatter plots). To standardize analysis of mean TAb titer across treatment groups with different total exposure to elosulfase alfa, a mean TAb titer was derived for each patient using a limited number of shared time points between the treatment arms. Mean TAb titers were derived from visit weeks 12, 24, 48, 72, and 96 of the QW-QW and QOW-QOW treatment groups and visit weeks 36, 48, 72, 96, and 120 of the PBO-QW and PBO-QOW groups using the ITT population. Therefore, each mean titer represents antibody levels after 96 total weeks of treatment (accounting for the 24 weeks of placebo treatment in the PBO-QW and PBO-QOW groups). Mean titers were then used to define quartile groupings, which were used across all analyses.

## RESULTS

The baseline demographic characteristics and primary tolerability and efficacy outcomes of MOR-005 have been reported elsewhere.<sup>18</sup> In brief, patients entering MOR-005 were 50.3% female, with a median age at enrollment of 11.7 years. Improvements from baseline in the primary efficacy measure (6MWT) observed in MOR-004 were maintained through week 120. Safety profile findings were consistent with those in MOR-004, and no new safety signals were identified.<sup>18</sup>

### Long-Term Immunogenicity Profile

In MOR-004, all patients treated with elosulfase alfa developed anti-elosulfase alfa TAb by week 16.<sup>17</sup> All patients treated with elosulfase alfa in MOR-004 remained positive for TAb throughout MOR-005 (Table I). Patients who received placebo in MOR-004 initiated elosulfase alfa treatment on entering MOR-005, and all patients in this group tested

positive for TAb after 12 weeks of elosulfase alfa treatment (Table I). From study week 36 through week 120, all patients remained TAb positive, with similar mean titer values across all treatment groups (Figures 1A, 1B; see Supplemental Table I in the online version at <http://dx.doi.org/10.1016/j.clinthera.2016.11.017>). There were no apparent differences in TAb titer between the ITT (Figure 1A) and MPP (Figure 1B) populations. NABs, defined as antibodies capable of inhibiting elosulfase alfa from binding to the CI-M6PR in vitro, were detected in most patients treated with elosulfase alfa in MOR-004, and most patients from the placebo group of MOR-004 had NAB positivity after 12 weeks of elosulfase alfa treatment in MOR-005 (Table I). The proportion of patients with NAB positivity peaked at 85.9% (146 of 170) at week 36 and then steadily declined throughout the remainder of the study (Table I). At week 120, 66.0% of patients (99 of 150) tested positive for NAB (Table I). At week 120, NAB incidence was similar across treatment groups, and there were no apparent differences between the ITT (Figure 1C) and MPP (Figure 1D) populations.

### Immunogenicity and Pharmacodynamics and Efficacy

Immunogenicity data from the MPP population were analyzed for association with effort-based measures of efficacy (6MWT and 3MSCT). Immunogenicity data from the ITT population were analyzed for association with changes in uKS (a measure of pharmacodynamic effect) because normalized uKS is not influenced by the variables (joint abnormalities, neurologic problems, patient motivation, and orthopedic surgeries) that may influence effort-based measures of efficacy. Both the MPP and ITT populations were divided into patients who received elosulfase alfa and patients who received placebo in MOR-004.

Despite the sustained presence of ADAs, initial improvements in endurance and reductions in uKS reported in MOR-004 were maintained in this extension study over a total of 120 weeks.<sup>18</sup> Patients who received elosulfase alfa in MOR-004 exhibited an initial rapid decline in normalized uKS, followed by a continuous gradual decline through week 120; reductions in uKS were similar regardless of TAb quartile (Figure 2A). Patients who received placebo in MOR-004 exhibited a similar rapid initial decline in normalized uKS after 12 weeks of elosulfase alfa

Table I. Incidence of anti-elosulfase alfa antibody positivity by week (intention-to-treat population).

| Antibody Positivity        | PBO-QOW<br>(n = 29) | PBO-QW<br>(n = 29) | QOW-QOW<br>(n = 59) | QW-QW<br>(n = 58) | Total<br>(n = 175)          |
|----------------------------|---------------------|--------------------|---------------------|-------------------|-----------------------------|
| TAb Titer Positive*        |                     |                    |                     |                   |                             |
| Baseline                   | 6/29 (20.7)         | 6/29 (20.7)        | 15/59 (25.4)        | 9/58 (15.5)       | 24/117 (20.5) <sup>†</sup>  |
| Week 12                    | 6/29 (20.7)         | 8/29 (27.6)        | 57/58 (98.3)        | 56/56 (100.0)     | 113/114 (99.1) <sup>†</sup> |
| Week 24 (MOR-005 week 0)   | 2/29 (6.9)          | 6/29 (20.7)        | 59/59 (100.0)       | 54/55 (98.2)      | 113/114 (99.1) <sup>†</sup> |
| Week 36 (MOR-005 week 12)  | 29/29 (100.0)       | 28/28 (100.0)      | 58/58 (100.0)       | 55/55 (100.0)     | 170/170 (100)               |
| Week 48 (MOR-005 week 24)  | 29/29 (100.0)       | 27/27 (100.0)      | 58/58 (100.0)       | 54/54 (100.0)     | 168/168 (100)               |
| Week 72 (MOR-005 week 48)  | 28/28 (100.0)       | 28/28 (100.0)      | 58/58 (100.0)       | 54/54 (100.0)     | 168/168 (100)               |
| Week 96 (MOR-005 week 72)  | 27/27 (100.0)       | 26/26 (100.0)      | 56/56 (100.0)       | 53/53 (100.0)     | 162/162 (100)               |
| Week 120 (MOR-005 week 96) | 24/24 (100.0)       | 27/27 (100.0)      | 49/49 (100.0)       | 50/50 (100.0)     | 150/150 (100)               |
| Overall                    | 29/29 (100.0)       | 28/28 (100.0)      | 59/59 (100.0)       | 57/58 (98.3)      | 173/174 (99.4)              |
| NAb Positive Screening     |                     |                    |                     |                   |                             |
| Baseline                   | 0/29                | 0/29               | 0/59                | 0/58              | 0/117 <sup>†</sup>          |
| Week 12                    | 0/29                | 3/29 (10.3)        | 48/58 (82.8)        | 49/56 (87.5)      | 97/114 (85.1) <sup>†</sup>  |
| Week 24 (MOR-005 week 0)   | 1/29 (3.4)          | 1/29 (3.4)         | 47/59 (79.7)        | 48/55 (87.3)      | 95/114 (83.3) <sup>†</sup>  |
| Week 36 (MOR-005 week 12)  | 26/29 (89.7)        | 23/28 (82.1)       | 47/58 (81.0)        | 50/55 (90.9)      | 146/170 (85.9)              |
| Week 48 (MOR-005 week 24)  | 22/29 (75.9)        | 21/27 (77.8)       | 44/58 (75.9)        | 42/54 (77.8)      | 129/168 (76.8)              |
| Week 72 (MOR-005 week 48)  | 23/28 (82.1)        | 17/28 (60.7)       | 43/58 (74.1)        | 41/54 (75.9)      | 124/168 (73.8)              |
| Week 96 (MOR-005 week 72)  | 21/27 (77.8)        | 15/26 (57.7)       | 37/56 (66.1)        | 38/53 (71.7)      | 111/162 (68.5)              |
| Week 120 (MOR-005 week 96) | 16/24 (66.7)        | 16/27 (59.3)       | 29/49 (59.2)        | 38/50 (76.0)      | 99/150 (66.0)               |
| Overall                    | 29/29 (100.0)       | 27/28 (96.4)       | 59/59 (100.0)       | 57/58 (98.3)      | 172/174 (98.9)              |
| IgE Positive               |                     |                    |                     |                   |                             |
| Baseline                   | 0/29                | 1/29 (3.4)         | 0/59                | 2/58 (3.4)        | 2/117 (1.7) <sup>†</sup>    |
| Week 12                    | 0/29                | 0/29               | 0/58                | 1/57 (1.8)        | 1/115 (0.9) <sup>†</sup>    |
| Week 24 (MOR-005 week 0)   | 0/29                | 0/28               | 0/58                | 2/55 (3.6)        | 2/113 (1.8) <sup>†</sup>    |
| Week 36 (MOR-005 week 12)  | 0/29                | 1/28 (3.6)         | 0/58                | 0/55              | 1/170 (0.6)                 |
| Week 48 (MOR-005 week 24)  | 0/29                | 0/27               | 1/58 (1.7)          | 1/54 (1.9)        | 2/168 (1.2)                 |
| Week 72 (MOR-005 week 48)  | 0/28                | 0/28               | 2/58 (3.4)          | 0/54              | 2/168 (1.2)                 |
| Week 96 (MOR-005 week 72)  | 1/27 (3.7)          | 0/26               | 2/55 (3.6)          | 0/52              | 3/160 (1.9)                 |
| Week 120 (MOR-005 week 96) | 1/24 (4.2)          | 0/27               | 1/49 (2.0)          | 0/49              | 2/149 (1.3)                 |
| Overall                    | 1/29 (3.4)          | 1/28 (3.6)         | 8/59 (13.6)         | 6/58 (10.3)       | 16/174 (9.2)                |

Values are no./study visit total (%).

IgE = immunoglobulin E; NAb = neutralizing antibody; PBO = placebo; QOW = every other week; QW = every week; TAb = total antibody.

\*Tab titer result equal to or greater than minimum required dilution (1:10).

<sup>†</sup>Calculation of total incidence from baseline, week 12, and week 24 visits excluded PBO-QOW and PBO-QW treatment groups because these groups were not on active study drug during this time.

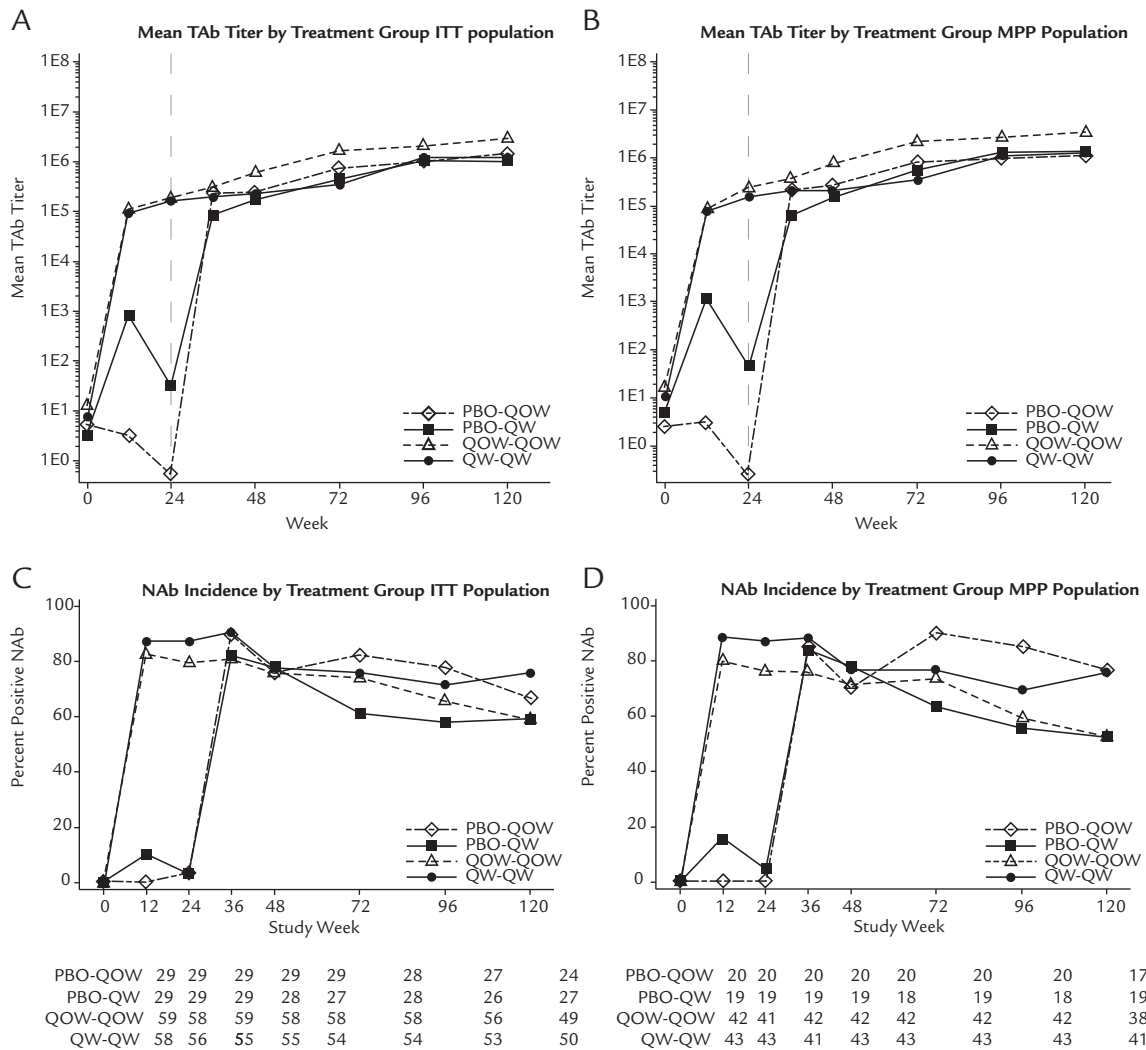


**Figure 1.** Mean antitoxin total antibody (TAB) titers and neutralizing antibody (NAb) incidence over 120 weeks in patients who received elosulfase alfa. (A) Mean TAB titer by treatment group for the intention-to-treat (ITT) population is presented by study week; patients who received placebo in MOR-004 and then were re-randomized to receive elosulfase alfa every week (PBO-QW) or every other week (PBO-QOW) began treatment at study week 24. (B) Mean TAB titer by treatment group for the modified per-protocol (MPP) population is presented by study week. (C) NAb incidence by treatment group for the ITT population is presented by study week with PBO-QOW and PBO-QW groups beginning treatment at study week 24. Numbers of patients in each treatment group at each study week are presented below. (D) NAb incidence by treatment group for the MPP population is presented by study week. Numbers of patients in each treatment group at each study week are presented below. QW-QW = every week.

treatment to levels comparable with those of continuously treated patients by study week 72; reductions were similar regardless of TAB quartile (Figure 2B). At week 120, mean (SE) normalized uKS had decreased from baseline by 59.4% (1.8%). Both effort-based

efficacy measures (6MWT and 3MSCT) indicated improvements over 120 weeks.<sup>18</sup> The mean (SE) change from baseline to week 120 in the primary efficacy measure of 6MWT was 31.7 (6.8) meters, and the mean (SE) change from baseline in the secondary

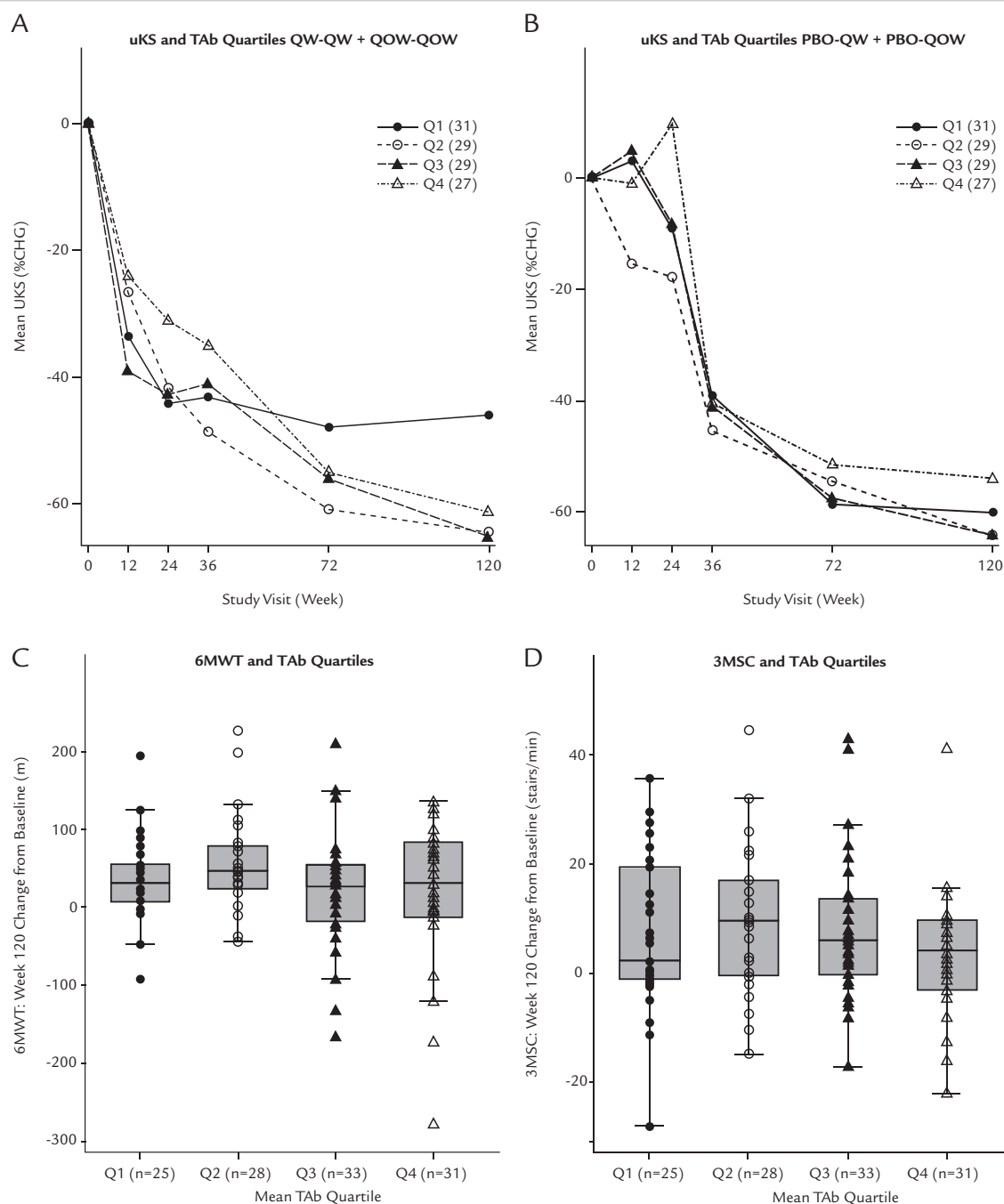


**Figure 2.** Antidrug total antibody (Tab) titer and changes in pharmacodynamic and efficacy measures. (A) Changes in urine keratan sulfate (uKS) levels by TAB quartile (Q) over 120 weeks for the patients randomized to receive elosulfase alfa in MOR-004 who continued to receive their assigned dose of 2.0 mg/kg every week (QW-QW) or every other week (QOW-QOW). (B) Changes in uKS levels by TAB Q over 120 study weeks for the patients who received placebo in MOR-004 and then were re-randomized to receive elosulfase alfa every week (PBO-QW) or every other week (PBO-QOW). (C) Change from baseline to week 120 in meters for 6-minute walk test (6MWT) distance in modified per-protocol (MPP) patients by TAB Q. (D) Change from baseline to week 120 in stairs per minute climbed by MPP patients during the 3-minute stair climb test (3MSCT) by TAB Q. CHG = change.

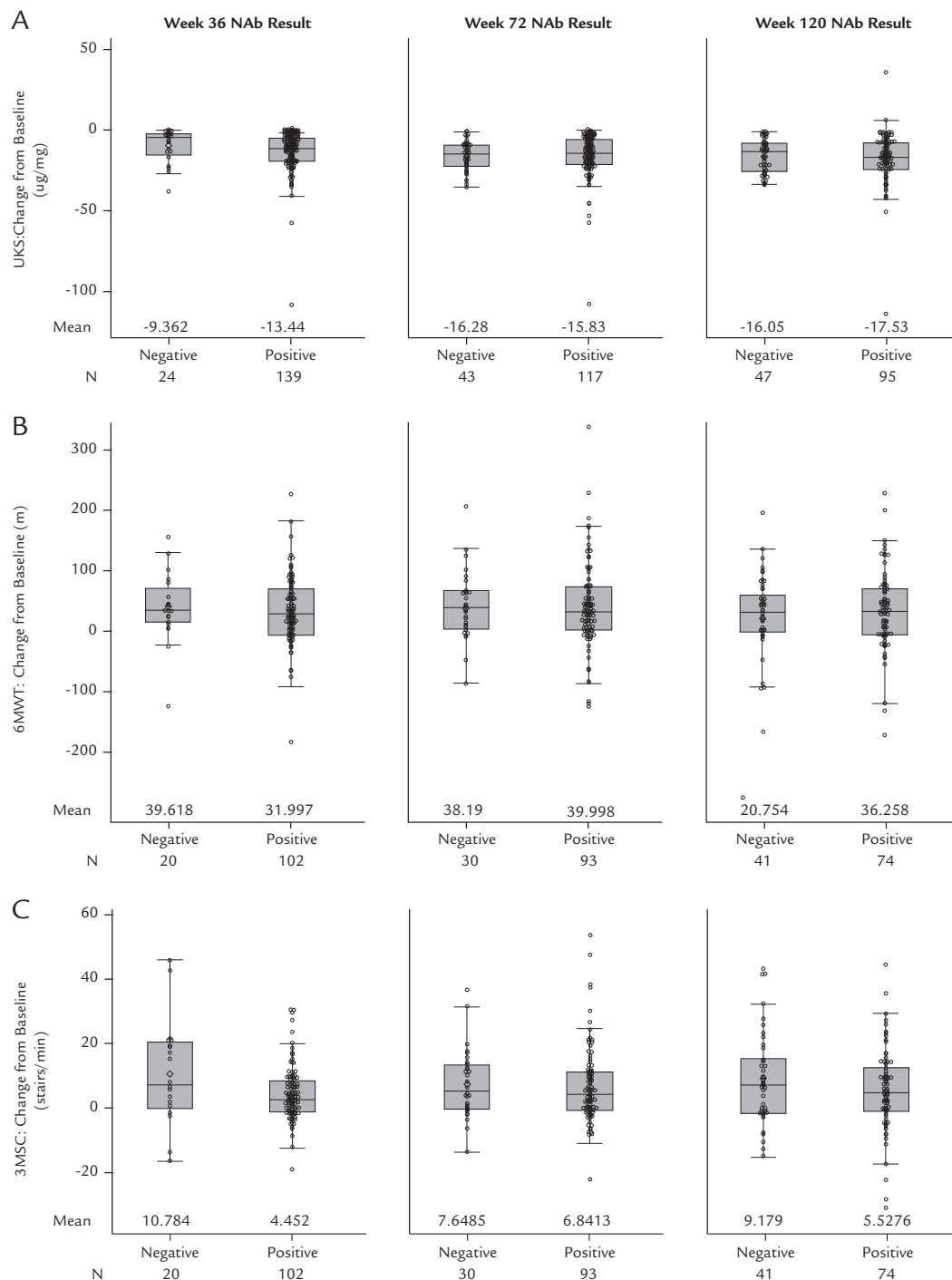


**Figure 3.** Neutralizing antibody (Nab) incidence and changes in pharmacodynamic and efficacy measures at study weeks 36, 72, and 120. (A) Mean change from baseline in urine keratan sulfate (uKS) levels in intention-to-treat patients who tested positive or negative for Nab at study weeks 36, 72 and 120. (B) Mean change from baseline in meters for 6-minute walk test (6MWT) distance in modified per-protocol (MPP) patients who tested positive or negative for Nab at study weeks 36, 72, and 120. (C) Mean change from baseline in stairs per minute climbed for 3-minute stair climb test (3MSCT) in MPP patients who tested positive or negative for Nab at study weeks 36, 72, and 120.

efficacy measure of 3MSCT was 6.8 (1.3) stairs/min.<sup>18</sup> No association between change from baseline to week 120 and TAB quartile was observed for either measure (Figures 2C, 2D).

Potential associations between NAb positivity and pharmacodynamic and efficacy measures were assessed at weeks 36, 72, and 120. At each time point, patients were stratified by NAb status and were evaluated for associations with changes from baseline in uKS, 6MWT, and 3MSCT (Figure 3). At week 120, the mean change from baseline in normalized uKS, 6MWT, and 3MSCT in patients who tested positive for NAb was 17.5, 36.3, and 5.5, respectively, compared with 16.1, 20.8, and 9.2 in patients who tested negative for NAb (Figure 3). Importantly, no differences were observed between groups in the change from baseline in uKS (Figure 3A), 6MWT (Figure 3B), or 3MSCT (Figure 3C) at any time point.

### Immunogenicity and Tolerability

As reported previously, all elosulfase alfa-treated patients reported at least 1 AE during MOR-005, and 0.5% (81 of 15,141) of administered infusions were interrupted or discontinued because of an AE requiring medical intervention.<sup>18</sup> Most AEs were mild-to-moderate infusion-associated reactions that were generally manageable with symptomatic treatment and/or infusion rate modifications, and <3% of patients permanently discontinued the study drug.<sup>18</sup>

Eighteen patients (18 of 173, 10.4%) on the study drug were identified as having an anaphylaxis event over the 120 weeks of MOR-004 and the MOR-005 extension study (1 additional event occurred in a patient on placebo). Most anaphylaxis incidents were mild to moderate in severity. A total of 16 patients (16 of 174, 9.2%) tested positive for elosulfase alfa-specific IgE at the indicated study visits (Table I); however, IgE positivity was not consistently associated with either the occurrence or severity of anaphylaxis because only 3 of these 18 patients (11.1%) were IgE positive at or near the time of the anaphylaxis event. Importantly, anaphylaxis resolved in all patients, and no patients discontinued treatment due to anaphylaxis. In addition, there was no apparent association between mean TAB titers and the presence/absence of anaphylaxis events, HAEs, or HAE severity (see Supplemental Figures 1 and 2 in the online version at <http://dx.doi.org/10.1016/j.clinthera.2016.11.017>).

### DISCUSSION

The data presented here indicate a continued benefit of elosulfase alfa over 120 weeks of treatment, despite a sustained ADA response. Consistent with previous findings,<sup>16</sup> antibodies to elosulfase alfa developed in all patients after 12 to 16 weeks of treatment, and patients remained positive for ADAs throughout the present study. Importantly, higher TAB titers were not associated with worsened pharmacodynamic effect or efficacy outcomes. Patients in the highest TAB quartile exhibited similar changes from baseline in mean 6MWT, 3MSCT, and uKS levels compared with patients in the lower TAB quartiles. Nearly all patients (>98%) tested positive for NAb at least once during the course of the study. The incidence of NAb positivity was similar across all treatment groups, peaked after 12 weeks of elosulfase alfa treatment, and then steadily declined throughout the remainder of the study. However, NAb positivity was not associated with worsened pharmacodynamic or efficacy outcomes in this study. Of note, a proportion of patients tested positive for anti-elosulfase alfa before having been exposed to the drug (Table I). All samples from placebo-treated patients testing positive for TAB and/or NAb were confirmed as specific to elosulfase alfa. The observance of preexisting or cross-reacting antibodies is not uncommon in biological therapies and has been previously reported for other ERTs.<sup>22</sup>

The rate of anaphylaxis remained relatively low throughout the study and was reported in 10.4% of patients over the 120 weeks of treatment. There was no consistent correlation observed between patients who tested positive for antidrug IgE and either the incidence or severity of anaphylaxis, and only 2 of 18 patients tested positive for elosulfase alfa-specific IgE at or near the time of the event. Importantly, all patients who experienced anaphylaxis were successfully re-challenged, and none were permanently discontinued from treatment, suggesting that these responses were likely mediated via immune complexes, rather than via IgE. In addition, no association was observed between mean TAB titer for patients who experienced HAEs or anaphylaxis versus those who did not, and TAB titer was not associated with HAE severity.

Although virtually all commercially available ERTs elicit ADA responses, both the prevalence and clinical impact of ADA formation vary among ERTs. In some

cases, sustained, high-titer antibody responses can significantly alter the efficacy of ERT. For instance, the efficacy of alglucosidase alfa (for treatment of Pompe disease) is significantly reduced in patients with high sustained ADA titers, particularly in patients without residual native protein (cross-reactive immunologic material-negative patients).<sup>15,23,24</sup> In other cases, ADA responses are not robust or sustained, may decrease over time, and/or do not appear to significantly reduce drug efficacy.<sup>15,23–25</sup> The reason that ADAs are associated with diminished efficacy in some ERTs, but have little to no impact on others, is not well understood.

In the case of elosulfase alfa, the apparent lack of association of ADAs with worsened tolerability or efficacy outcomes may be at least partially associated with the rapid uptake of the drug from the plasma into target cells via the mannose-6-phosphate receptor.<sup>17,26</sup> Mean plasma half-lives of distinct ERTs can vary widely both within patient populations and across indications. For example, galsulfase (for treatment of Maroteaux-Lamy syndrome) exhibits a mean plasma half-life of 26 minutes, whereas laronidase (for treatment of Hurler-Scheie syndrome) has a mean plasma half-life ranging from 1.5 to 3.6 hours after repeated administration.<sup>27,28</sup> Elosulfase alfa exhibits a mean plasma half-life of 36 minutes after repeat administration, followed by a mean intracellular half-life of 5 to 7 days in human fibroblasts.<sup>12,26,29</sup> This rapid uptake may limit the drug's exposure to antibodies in the plasma and, in turn, reduce the formation of immune complexes and their downstream effects. These results are similar to those found with laronidase and agalsidase beta (for treatment of Fabry disease) and highlights that both the risk and impact of immunogenicity are not universally comparable across ERT disease indications.<sup>30,31</sup>

## CONCLUSIONS

At the final time point of week 120, all patients had developed antibodies specific to elosulfase alfa. However, higher TAB titers were not associated with worsened efficacy measures (6MWT, 3MSCT), pharmacodynamic effect (uKS), or safety profile outcomes. The proportion of patients with NAb positivity peaked at week 36 and then steadily declined throughout the remainder of the study. There was no association detected between NAb positivity and

efficacy, pharmacodynamics, or safety profile measures. Anaphylactic events were experienced by 10.4% of patients on the study drug; however, neither the number nor severity of these events was consistently associated with the presence of anti-elosulfase alfa IgE or higher TAB titers. These data are consistent with those from previously reported studies and indicate that elosulfase alfa is an efficacious and well-tolerated treatment for patients with Morquio A syndrome.<sup>17</sup>

## AUTHOR CONTRIBUTIONS

B. Long wrote the manuscript, analyzed data and created figures; T. Tompkins analyzed data and created figures; C. Decker contributed to study design and collected and analyzed clinical data; S. Khan analyzed clinical safety data; P. Slasor collected data, provided statistical analysis and created figures and tables; P. Harmatz analyzed and interpreted clinical data; C.A. O'Neill analyzed and interpreted data and B. Schweighardt analyzed and interpreted data and contributed to figure and table creation.

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## CONFLICT OF INTEREST

B. Long, T. Tompkins, C. Decker, L. Jesaitis, S. Khan, P. Slasor, C.A. O'Neill, and B. Schweighardt are employees and stockholders of BioMarin Pharmaceutical Inc. P. Harmatz is a consultant for BioMarin, Shire, Alexion, PTC, Ciesi, Armagen, Genzyme, and Inventiva; has participated in symposia sponsored by BioMarin, Shire, Genzyme, PTC, and Alexion; has received grant/research support from BioMarin, Alexion/Enobia, Shire, Sanofi-Genzyme, and Armagen; and has received honoraria from BioMarin, Alexion/Enobia, Shire, Sanofi-Genzyme, PTC, Ciesi, and Inventiva.

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

## SUPPLEMENTARY MATERIAL

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.clinthera.2016.11.017>.

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## Clinical Therapeutics

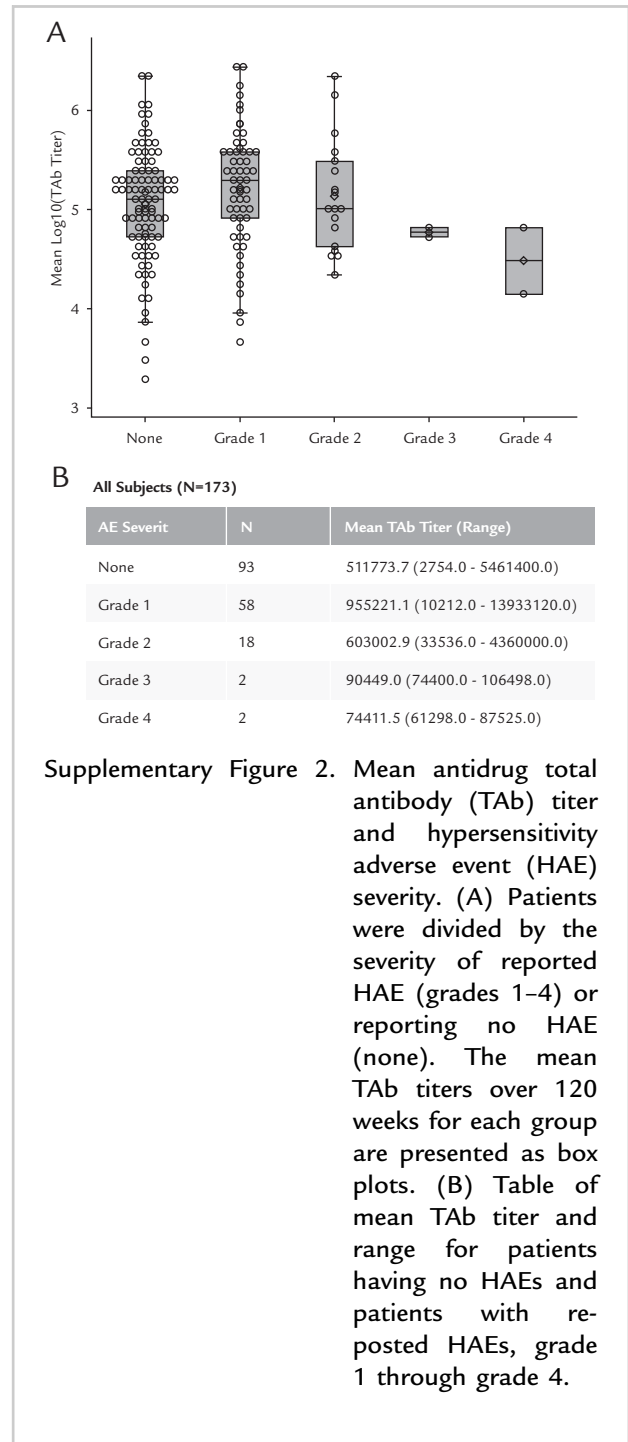
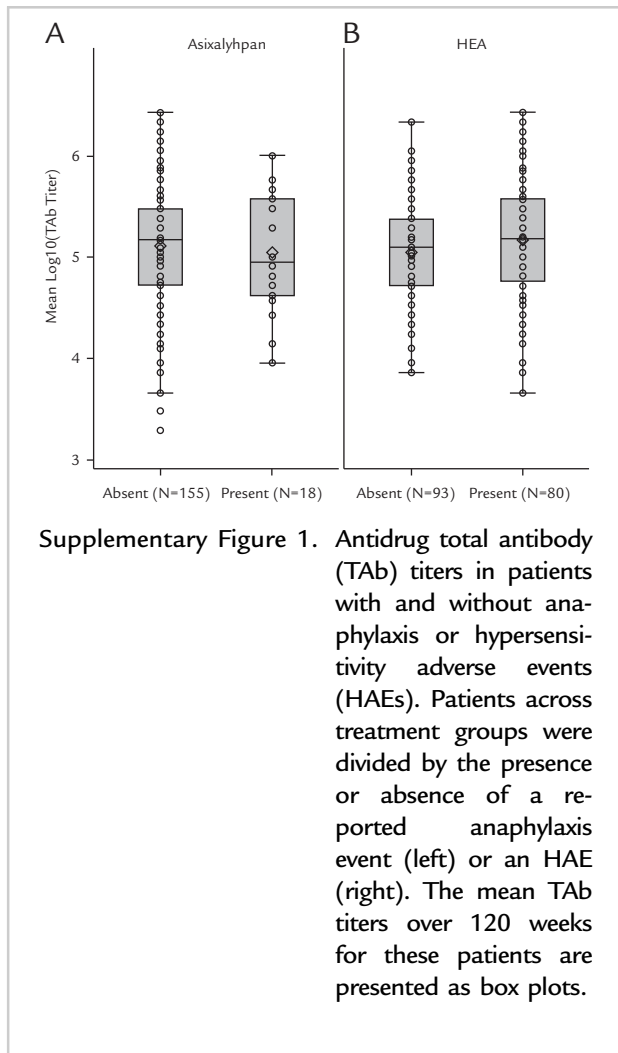
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**Address correspondence to:** Brian Long, PhD, BioMarin Pharmaceutical Inc, 105 Digital Drive, Novato, CA94949. E-mail: Brian.Long@bmrn.com

## SUPPLEMENTARY MATERIAL

Figs. 1 and 2, Table I



Supplemental Table I. Antidrug total antibody (TAB) titer by study visit week.

| Study Visit            | PBO-QOW<br>(n = 29) | PBO-QW<br>(n = 29)  | QOW-QOW<br>(n = 59) | QW-QW<br>(n = 58)   |
|------------------------|---------------------|---------------------|---------------------|---------------------|
| Baseline               |                     |                     |                     |                     |
| No.                    | 29                  | 29                  | 59                  | 58                  |
| Mean (SD)              | 5 (17.4)            | 3 (8.0)             | 14 (50.3)           | 8 (37.3)            |
| Median                 | 0                   | 0                   | 0                   | 0                   |
| 25th, 75th percentiles | 0, 0                | 0, 0                | 0, 0                | 0, 0                |
| Minimum, maximum       | 0, 90               | 0, 30               | 0, 270              | 0, 270              |
| Week 12                |                     |                     |                     |                     |
| No.                    | 29                  | 29                  | 58                  | 56                  |
| Mean (SD)              | 3 (8.0)             | 758 (4066.1)        | 117,229 (251,548.7) | 94,734 (134,114.9)  |
| Median                 | 0                   | 0                   | 65,600              | 65,600              |
| 25th, 75th percentiles | 0, 0                | 0, 5                | 7290, 197,000       | 21,900, 65,600      |
| Minimum, maximum       | 0, 30               | 0, 21,900           | 0, 1,770,000        | 90, 590,000         |
| Week 24                |                     |                     |                     |                     |
| No.                    | 29                  | 29                  | 59                  | 55                  |
| Mean (SD)              | 1 (2.0)             | 33 (150.5)          | 190,901 (343,722.0) | 167,737 (398,716.8) |
| Median                 | 0                   | 0                   | 65,600              | 65,600              |
| 25th, 75th percentiles | 0, 0                | 0, 0                | 21,900, 197,000     | 21,900, 65,600      |
| Minimum, maximum       | 0, 10               | 0, 810              | 810, 1,770,000      | 0, 1,770,000        |
| Week 36                |                     |                     |                     |                     |
| No.                    | 29                  | 28                  | 58                  | 55                  |
| Mean (SD)              | 235,513 (458,865.4) | 79,691 (120,993.3)  | 301,955 (443,744.7) | 214,960 (404,743.1) |
| Median                 | 65,600              | 21,900              | 197,000             | 65,600              |
| 25th, 75th percentiles | 21,900, 197,000     | 21,900, 65,600      | 65,600, 197,000     | 21,900, 197,000     |
| Minimum, maximum       | 270, 1,770,000      | 7290, 590,000       | 2430, 1,770,000     | 2430, 1,770,000     |
| Week 48                |                     |                     |                     |                     |
| No.                    | 29                  | 27                  | 58                  | 54                  |
| Mean (SD)              | 239,475 (229,281.9) | 169,036 (195,471.0) | 637,422 (1,329,600) | 232,300 (353,248.1) |
| Median                 | 197,000             | 65,600              | 197,000             | 197,000             |
| 25th, 75th percentiles | 65,600, 590,000     | 21,900, 197,000     | 65,600, 590,000     | 21,900, 197,000     |
| Minimum, maximum       | 7290, 590,000       | 810, 590,000        | 2430, 5,310,000     | 810, 1,770,000      |

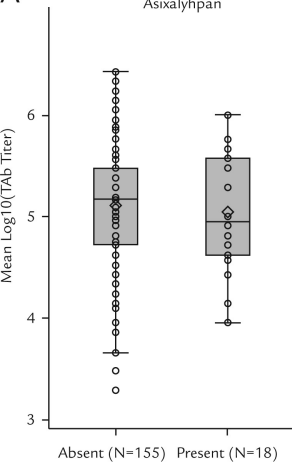
(continued)

Supplemental Table I. (continued).

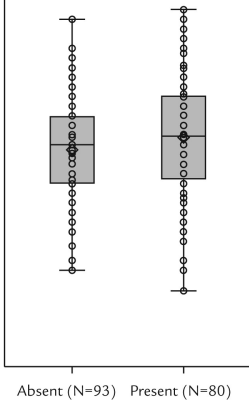
| Study Visit            | PBO-QOW<br>(n = 29)   | PBO-QW<br>(n = 29)    | QOW-QOW<br>(n = 59)   | QW-QW<br>(n = 58)     |
|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Week 72                |                       |                       |                       |                       |
| No.                    | 28                    | 28                    | 58                    | 54                    |
| Mean (SD)              | 729,036 (1,368,715)   | 430,490 (1,021,834)   | 1,741,686 (6,300,172) | 373,463 (499,119.6)   |
| Median                 | 197,000               | 131,300               | 590,000               | 197,000               |
| 25th, 75th percentiles | 65,600, 590,000       | 65,600, 393,500       | 65,600, 1,770,000     | 65,600, 590,000       |
| Minimum, maximum       | 21,900, 5,310,000     | 810, 5,310,000        | 2430, 47,800,000      | 7290, 1,770,000       |
| Week 96                |                       |                       |                       |                       |
| No.                    | 27                    | 26                    | 56                    | 53                    |
| Mean (SD)              | 1,030,381 (1,410,478) | 1,035,870 (3,078,997) | 2,200,715 (4,095,498) | 1,236,296 (3,123,980) |
| Median                 | 590,000               | 197,000               | 590,000               | 197,000               |
| 25th, 75th percentiles | 197,000, 1,770,000    | 65,600, 590,000       | 197,000, 1,770,000    | 65,600, 590,000       |
| Minimum, maximum       | 21,900, 5,310,000     | 2430, 15,900,000      | 2430, 15,900,000      | 21,900, 15,900,000    |
| Week 120               |                       |                       |                       |                       |
| No.                    | 24                    | 27                    | 49                    | 50                    |
| Mean (SD)              | 1,376,742 (1,659,284) | 985,382 (1,621,095)   | 2,981,358 (7,324,298) | 1,234,148 (2,618,483) |
| Median                 | 590,000               | 590,000               | 590,000               | 393,500               |
| 25th, 75th percentiles | 197,000, 1,770,000    | 65,600, 590,000       | 197,000, 1,770,000    | 197,000, 590,000      |
| Minimum, maximum       | 65,600, 5,310,000     | 2430, 5,310,000       | 2430, 47,800,000      | 810, 15,900,000       |

**A**

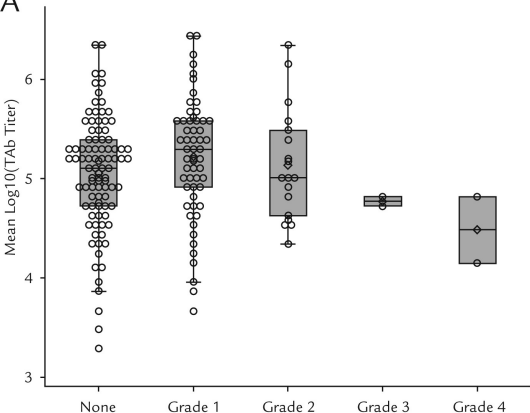
Asixalyhpan

**B**

HEA



A



B

All Subjects (N=173)

| AE Severit | N  | Mean TAB Titer (Range)          |
|------------|----|---------------------------------|
| None       | 93 | 511773.7 (2754.0 - 5461400.0)   |
| Grade 1    | 58 | 955221.1 (10212.0 - 13933120.0) |
| Grade 2    | 18 | 603002.9 (33536.0 - 4360000.0)  |
| Grade 3    | 2  | 90449.0 (74400.0 - 106498.0)    |
| Grade 4    | 2  | 74411.5 (61298.0 - 87525.0)     |