Reveglucosidase alfa (BMN 701), an IGF 2 Tagged rhAcid α-Glucosidase, Improves Respiratory Functional Parameters in a Murine Model of Pompe Disease

Prepared for submission to:

*The Journal of Pharmacology and Experimental Therapeutics*

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The number of pages:

Text: 27

Tables: 7

Figures: 2

References: 6

Word count:

Abstract: 249

Introduction: 734

Discussion: 1488

List of Nonstandard Abbreviations
BMN 701 reveglucosidase alfa

CI-M6PR Cation independent mannose-6-phosphate receptor

ERT enzyme replacement therapy

GAA acid alpha (α)-glucosidase

IGF2 insulin-like growth factor 2

IGF2R insulin-like growth factor 2 receptor

LFB Luxol Fast blue

LOPD late-onset Pompe disease

M6P mannose-6-phosphate

MEP maximal expiratory pressure

MIP maximal inspiratory pressure

MV minute volume

PAS Periodic acid-Schiff

PEF peak expiratory flow

PIF peak inspiratory flow

rhGAA recombinant human acid alpha(α)-glucosidase; aglucosidase alfa

RR respiratory rate

T_e expiratory time
$T_i$ inspiratory time

TV tidal volume

**Recommended section assignment**: Drug Discovery and Translational Medicine
ABSTRACT

Pompe disease is a rare neuromuscular disorder caused by an acid $\alpha$-glucosidase (GAA) deficiency resulting in glycogen accumulation in muscle, leading to myopathy and respiratory weakness. Reveglucosidase alfa (BMN 701), is an insulin-like growth factor 2 (IGF2) tagged recombinant human acid GAA (rhGAA) that enhances rhGAA cellular uptake via a glycosylation independent IGF2-binding region of the cation-independent mannose-6-phosphate receptor (CI-MPR). These studies evaluated the effects of reveglucosidase alfa treatment on glycogen clearance in muscle relative to rhGAA as well as changes in respiratory function and glycogen clearance in respiratory related tissue in a Pompe mouse model ($\text{GAA}^{\text{tm1Rarb}}/\text{J}$). In a comparison of glycogen clearance in muscle with reveglucosidase alfa and rhGAA, reveglucosidase alfa was more effective than rhGAA with 2.8 to 4.7 lower $EC_{50}$ values, likely due to increased cellular uptake. The effect of weekly intravenous (IV) administration of reveglucosidase alfa on respiratory function was monitored in Pompe and wild type (WT) mice using whole body plethysmography. Over 12-weeks of 20 mg/kg reveglucosidase alfa treatment in Pompe mice, peak inspiratory flow (PIF) and peak expiratory flow (PEF) stabilized with no compensation in respiratory rate and inspiratory time during hypercapnic and recovery conditions compared to vehicle-treated Pompe mice. Dose related decreases in glycogen levels in both ambulatory and respiratory muscles generally correlated to changes in respiratory function. Improvement of murine PIF and PEF were similar in magnitude to increases in maximal inspiratory and expiratory pressure observed clinically in late onset Pompe patients treated with reveglucosidase alfa (Byrne et al, in preparation).
INTRODUCTION

Pompe disease is a rare progressive metabolic myopathy caused by a lack or deficiency of lysosomal acid alpha(α)-glucosidase (GAA) (van der Beek NA et al., 2006). This enzyme is responsible for the degradation of alpha-1,4 and alpha-1,6 linkages in glycogen (Raben N et al., 2002). Consequently, a deficiency of this enzyme results in the accumulation of glycogen within cellular lysosomes and cytoplasm, leading to tissue damage with the most severe pathology in the skeletal and respiratory muscles (Raben N et al., 2002, van der Ploeg et al, 2008).

Clinically, Pompe disease is a heterogeneous disorder with a continuous clinical spectrum occurring from infancy through adulthood, affecting a range of target organs and resulting in varying degrees of clinical severity (Di Rocco M et al., 2007; Schoser B et al., 2008; van der Ploeg AT and Reuser AJ, 2008). Patients with late-onset Pompe disease (LOPD) may present with progressive proximal myopathy at any age, with or without respiratory failure and cardiac symptoms (van der Beek NA et al., 2006). Diminished respiratory function is due to diaphragmatic and accessory respiratory muscle weakness and can be the first obvious clinical manifestation of LOPD (Di Rocco M et al., 2007, Mellies and Lofaso, 2008). These patients present with frequent respiratory infections, respiratory distress, orthopnea, sleep apnea, impaired cough, somnolence, and morning headaches. As the disease progresses, assisted ventilation becomes necessary. Evaluation of a limited number of patients with LOPD using both lung MRI and respiratory function assessments implicate the respiratory insufficiency mainly to diaphragmatic weakness (Gaeta et al, 2015, Wens et al, 2015). This inspiratory muscle weakness was pinpointed in LOPD patients with vital capacity in an upright position compared to vital capacity in a supine position (Mellies and Lofaso, 2008). Use of maximum
inspiratory/expiratory mouth pressure is a complementary non-invasive technique to evaluate inspiration and expiration muscle strength. Recent evidence suggests altered spinal and medullary respiratory neuron activity caused by glycogen accumulation may contribute to respiratory symptoms (Fuller DD et al., 2013) as extensively documented in the Pompe mouse model (Sidman RL et al., 2008).

The uptake of GAA into tissues is mediated via the bis-mannose 6-phosphorylated glycans of GAA binding to the cation-independent mannose-6-phosphate receptor (CI-MPR) (Kornfeld, 1990). The current standard of care for LOPD patients is enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA). rhGAA binds to the CI-MPR with lower affinity than reveglucosidase alfa, reducing uptake into the lysosome (McVie-Wiley et al, 2008, Tong et al, 1989) and may correlate with the need for relatively high rhGAA doses in the clinic. While rhGAA improves overall function as measured by 6-minute walk test and stabilizes forced vital capacity in LOPD patients after 78 weeks (van der Ploeg et al, 2010), efficacy may be suboptimal due to low amounts of the high affinity N-linked oligosaccharide bis-mannose 6-phosphate oligomannose 7 needed for efficient rhGAA uptake (Kakkis et al., 2008). Though ERT represents a major advancement in the treatment of these patients, effective clearance of skeletal muscle glycogen and associated neurons remains difficult.

Reveglucosidase alfa (BMN 701) is a novel fusion protein of a variant of insulin-like growth factor 2 (IGF2) and GAA and is being developed as a potential alternative treatment for Pompe disease (Maga et al, 2012; Figure 1). The glycosylation independent lysosomal targeting (GILT) tag utilizes the IGF2 receptor region of the CI-MPR for cellular uptake and subsequent trafficking to the lysosome, rather than depending on the glycosylation pattern of the GAA
protein and the M6P region (Maga JA et al., 2013). Because uptake is dependent on the IGF2 moiety rather than the mannose-based glycosylation pattern, reveglucosidase alfa has an increased \textit{in vitro} cellular uptake and a 26 times lower $K_{uptake}$ than untagged rhGAA. Using GAA$^{tmIRabn/J}$ (Pompe) mice with the same cellular and clinical characteristics of human LOPD (Raben N et al., 1998), reveglucosidase alfa was significantly more effective than untagged rhGAA in clearing glycogen from heart and skeletal muscles (Maga et al, 2012).

The overall objectives of these experiments using the Pompe mouse were: 1) to compare the relative glycogen clearance of reveglucosidase alfa with rhGAA; 2) assess the longitudinal effects of reveglucosidase alfa on the respiratory function of treated animals following 4 weekly (Phase 1) and 12 weekly doses of reveglucosidase alfa (Phase 2); and 3) assess glycogen clearance in respiratory and skeletal muscles and in some areas of CNS relative to the natural disease progression of respiratory function in the Pompe mouse model.

**MATERIALS AND METHODS**

**Study Drugs**

Reveglucosidase alfa is an IGF2 tagged rhGAA (Figure 1; Maga et al., 2013). Reveglucosidase alfa (MW 109 kDa) was prepared at nominal concentrations of 0.33, 1.0, or 1.7 mg/mL for dosing at 4, 12, or 20 mg/kg or 5 and 3 mg/mL for administration at 20 mg/kg and 12 mg/kg, respectively. Reveglucosidase alfa was stored at 2 to 8°C until dose administration (no longer than 24 hours). Reveglucosidase alfa is stable at 2-8°C for up to 4 weeks. Vehicle consisting of 3 mM citric acid, 22 mM sodium citrate dihydrate, 2% (w/v) D-mannitol, 0.5% (w/v) D-(+)-trehalose, 0.05% (w/v) poloxamer 188 in Sterile Water for Injection, USP, pH 6.05 to 6.20, was
prepared, filtered through a 0.22 µm PVDF filter and stored refrigerated at 2 to 8°C. A commercially available formulation of rhGAA (Myozyme® (algucosidase alfa); Genzyme Corporation, Framingham, MA) was supplied as a lyophilized cake or powder in glass vials. On each injection day, rhGAA (MW 118 kDa) was reconstituted with 10.3 mL sterile water for injection, and diluted with Myozyme® vehicle (210 mg mannitol, 0.5 mg Poysorbate-80, 9.9 mg sodium phosphate dibasic heptahydrate, 31.2 mg sodium phosphate monobasic monohydrate) to the appropriate concentration of 1.0, 1.7, or 5.0 mg/mL for administration at 12, 20, or 60 mg/kg, respectively. Upon reconstitution, vials containing rhGAA were inverted gently three times, stored at 2-8°C and dosed within 12 hours. Once reconstituted, rhGAA is stable for up to 24 hours at 2 to 8 °C (Genzyme Corporation 2014), and rhGAA drug substance can be stored at 6 to 10 °C for up to 4 weeks (European Medicines Agency 2006).

Diphenhydramine hydrochloride (DPH) was diluted to a concentration of 1 mg/mL in 0.9% Sodium Chloride for Injection, USP, filtered through a 0.22 µM PVDF filter, and stored at room temperature (20 to 25° C) until use. Based on previous observations of hypersensitivity due to administration of a heterologous protein, a 1 or 5 mg/kg dose of DPH was administered intraperitoneally approximately 10 to 15 minutes prior to each vehicle or reveglucosidase alfa dose administration to all animals from Week 3 until the end of dosing to reduce potential mortalities (Raben et al, 2003; Zhu et al, 2005).

**Study Animals**

The strains of mice (Jackson Laboratory, Bar Harbor, ME) used in these studies were wild-type (C57BL/6J; WT) and mice with a disrupted acid α-glucosidase gene (B6;129- GAAtm1Rabn/J; Pompe). The Pompe mice develop the same cellular and clinical characteristics as human adult
Pompe disease (Raben N et al., 1998). Animals were maintained in a 12-hour light/dark cycle, provided with fresh water and standard rodent chow *ad libitum* (Picolab Rodent 20 Diet; PMI Feeds Inc., or Lab Diet®; PMI Nutrition International, Inc, and DietGel® 76A (Clear H2O®, Portland, ME).

In the first comparison study evaluating glycogen clearance following administration of revglucosidase alfa or rhGAA, 4.5 to 5 month-old male and female mice were used to ensure high levels of glycogen storage in skeletal muscle (Pompe mice) or as comparative controls (WT mice). In the second and third study evaluating respiratory function, glycogen storage and histology changes, approximately 4-month old male and female Pompe and WT mice were used to ensure high levels of glycogen storage in diaphragmatic muscles after 15 or 14 weeks, respectively (Rabin, 1998). Animals were maintained in accordance with the U.S. Department of Agriculture Animal Welfare Act (United States Department of Agriculture, 2013), and the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The experimental protocols reflected generally accepted procedures for the testing of pharmaceutical compounds in animal models, and were reviewed and approved by the study site Institutional Animal Care and Use Committee prior to each study (International Council for Harmonization, 2007; Food and Drug Administration, 2015).

**In-study Observations**

In all phases of these comparisons, animals were observed for morbidity and mortality at least daily, with detailed clinical observations at least weekly. Additional monitoring during the respiratory function assessment was conducted during each hypercapnic exposure. At the time
of scheduled necropsy, or when found moribund, animals were humanely euthanized by carbon
dioxide inhalation.

**Glycogen Clearance following Administration of Reveglucosidase alfa or rhGAA for 4 Weeks**

Reveglucosidase alfa (4, 12, or 20 mg/kg), rhGAA (4, 20, or 60 mg/kg), or phosphate buffered
saline control (PBS) was administered to each animal once weekly for four weeks via
intravenous (IV) bolus injection to the tail vein.

Following a macroscopic assessment, the heart (left ventricle), quadriceps, diaphragm, psoas and
soleus muscles were collected, weighed, snap frozen in liquid nitrogen and stored at -60 to -90
°C prior to a quantitative analysis of glycogen-derived glucose. Muscles were homogenized in
buffer (0.2M NaOAc/0.5% NP40) on ice using ceramic spheres. Amyloglucosidase was added
to clarified lysates at 37°C to digest glycogen into glucose for subsequent colorimetric detection
(430 nm, SpectraMax) using a Peroxidase-Glucose Oxidase enzyme reaction system (Sigma-
Aldrich). Paired samples were also measured without amyloglucosidase to correct for
endogenous tissue glucose that was not in glycogen form at harvest. Glucose values were
extrapolated from a 6-point standard curve. The measured glucose concentration (mg/mL) was
proportional to the glycogen concentration of the sample and was converted to mg glycogen/g
tissue by adjusting for the homogenization step (5 µL buffer added per gram of tissue).

**Pharmacodynamics of Glycogen Clearance**

The effect of reveglucosidase alfa and rhGAA dose level on individual mouse muscle glycogen
levels were evaluated using Phoenix-WinNonlin classic PD modeling (Phoenix build version
6.4). The relationship between muscle glycogen levels and dose was best fit to an inhibitory
effect sigmoid E_0 model that yielded EC_{50} values for muscle glycogen reduction with dose level (inhibition of glycogen storage). The relationship between reveglucosidase alfa or rhGAA doses (C) and glycogen levels (E) was expressed as E = E_0 \left[1 - \frac{C^\gamma}{C^\gamma + IC_{50}^\gamma}\right] where E_0 in the basal glycogen level in the Pompe mouse muscle, EC_{50} is the dose required to produce 50% of the maximal glycogen reduction and \gamma is the slope factor (Hill coefficient) which determines the steepness of the glycogen reduction curve. Results were obtained for both reveglucosidase alfa and rhGAA in heart, diaphragm, quadriceps, psoas, and soleus muscles. Acceptability criteria were based on goodness of fit by visual inspection of diagnostic plots and by relative standard error of estimates on EC_{50} values. For the purposes of dose comparisons in mg/kg between rhGAA and reveglucosidase alfa, a correction factor based on differences in molecular weight was applied (Molecular weight rhGAA = 109 kDA, reveglucosidase alfa = 118 kDA). A structural comparison of rhGAA and reveglucosidase alfa is shown in Figure 1.

Respiratory Function, Glycogen Storage, and Histological Changes following Administration of Reveglucosidase alfa for 4 or 12 weeks

Dose Administration

PHASE 1, 4-Week Treatment

Reveglucosidase alfa (12 or 20 mg/kg; Pompe) or vehicle (control; WT and Pompe) was administered to each animal four times on Days 1, 8, 15, and 22 via intravenous (IV) bolus injection to the tail vein.

PHASE 2, 12-Week Treatment
Prior to treatment, a subset of untreated animals were monitored for respiratory changes at Weeks -12, -8 and -4 to ensure that the respiratory phenotype was present at study start. Animals were dosed weekly with vehicle or 20 mg/kg reveglucosidase alfa, IV for 12 weeks, and animals given 12 mg/kg/week reveglucosidase alfa were dosed weekly, IV for 4 weeks.

**Respiratory Function Assessment**

In Phase 1, a subset of each group of mice (n=5) was monitored for respiratory function. In Phase 2, all animals in each group (n=30) were monitored for respiratory function using a mouse plethysmograph chamber (Model PLY4211; Booth Medical Equipment, Alexander, AR). The number of animals evaluated in each group was estimated to be sufficient to assess an approximate 20% change in respiratory function. Data from male and female animals were combined for statistical analysis. Each chamber was supplied with a constant flow of oxygen at a rate of approximately 2 L/min for ≥1 hour of acclimation prior to initiating respiratory monitoring. After 1 hour of baseline data collection, the CO2 level within the chambers was increased to 7 ± 0.5% using a mixture of regulated CO2 and oxygen and respiratory monitoring was continued for a period of 15 minutes followed by monitoring under normoxic conditions for at least 45 minutes. Pulmonary endpoints included respiratory rate (RR), inspiratory time (T_i), tidal volume (TV), minute volume (MV; MV=TV*RR) and peak inspiratory flow (PIF). Expiration time (T_e) and peak expiratory flow (PEF) were also evaluated in Phase 2 animals. In Phase 1, animals were assessed for respiratory function at Weeks 1 and 4. In Phase 2, animals given 20 mg/kg reveglucosidase alfa were assessed for respiratory function at Weeks 1, 2, 4, 6, 8, 10 and 12 at least 48 hours following the weekly dose. Assessment of respiratory parameters emphasized PIF and PEF due to the increased duration and potential for differences in animal
growth. Animals given 12 mg/kg reveglucosidase alfa (Cohort 2) were evaluated during Week -12, -8, and -4 prior to dose administration and at Weeks 1 and 4 during treatment. All respiratory evaluations occurred at least 48 hours following the weekly dose.

Post-mortem Evaluation of Glycogen and Histological Changes in Skeletal Muscle,
Respiratory Muscle and Nervous Tissue

Selected tissues were snap frozen in liquid nitrogen and shipped frozen on dry ice for quantitative analysis of glycogen-derived glucose as described previously. In the Phase 2 animals treated for 12 weeks, additional tissues (intercostal muscles, brain, spinal cord and phrenic nerve) were collected for histological evaluation and glycogen content. Tissues from five animals, randomly selected, were evaluated per group. The ribs and intercostal muscles, brain, and spinal cord were collected, weighed and fixed in 10% neutral buffered formalin. The phrenic nerve was fixed in 2% methanol-free formaldehyde/2.5% glutaraldehyde for 48 hours, transferred to saline and stored at 2 to 8°C. Longitudinal and transverse sections of the intercostal muscles were embedded in paraffin and stained with H&E and LFB/PAS. Paraffin-embedded coronal sections of the brain were stained with hematoxylin and eosin (H&E) and Luxol Fast blue/Periodic acid-Schiff (LFB/PAS) to assess myelin (LFB) and glycogen content (PAS). Spinal cords were decalcified and at each level of the spinal cord (cervical, thoracic, lumbar), transverse and oblique sections were cut, embedded in paraffin and stained with H&E and LFB/PAS. Serial longitudinal and transverse sections of the phrenic nerve (left side) were cut, embedded in paraffin and stained with H&E and LFB/PAS. Two additional transverse sections were cut and embedded in resin and each stained with either PAS or Toluidine Blue for myelin. The ribs with intercostal muscles were decalcified. Analysis of glycogen content was
evaluated using a qualitative grading scale via high resolution light microscopy by a board certified veterinary pathologist (Tox Path Specialists, LLC; Frederick, MD). This five-point grade of increasing severity was 1 (Slight; barely exceeds normal limits), 2 (Minimal; more apparent than Grade 1, but unlikely to produce any structural or functional impairment), 3 (Mild; readily apparent in section but unlikely to have any functional significance), 4 (Moderate; prominent/conspicuous change with probable structural/functional significance) and 5 (Severe; extreme change; expected to have biologic significance).

**Pharmacokinetics (PK) Evaluation**

WT mice were administered a single dose of 4, 12 or 20 mg/kg reveglucosidase alfa via tail vein, IV bolus (18 animals/sex/group). Blood samples were collected as terminal cardiac punctures at pre-dose, 0.083, 0.5, 1, 2 and 4 hrs post-dose with \( N = 3 \) animals/gender/time point. Plasma reveglucosidase alfa concentrations were quantified using a bridging electrochemiluminescent method with an LOQ of 100 ng/mL. Briefly, 0.5 µg/mL ruthenium-labeled anti-rhGAA (affinity purified goat polyclonal) and 0.5 µg/mL biotin-labeled anti-IGF2 (R&D Systems, MAB792) were combined with K<sub>2</sub>EDTA plasma samples diluted 1:10 in buffer (Starting Block T20 (PBS), Thermo Scientific) and incubated for 1 hour before transfer to a blocked streptavidin assay plate (Meso Scale Discovery). After a 30 minute incubation, the plate was washed, 1x Read Buffer T (Meso Scale Discovery) was added, and the electrochemiluminescent signal read on an MSD Sector Imager 2400. Reveglucosidase alfa concentrations were extrapolated from a 9-point standard curve. Noncompartmental analysis was applied to the mean plasma reveglucosidase alfa concentration data for males, females and combined genders to estimate \( C_{\text{max}} \), \( T_{\text{max}} \), AUC,
t_{1/2}, CL and V_z. PK analysis was conducted using Phoenix WinNonlin (Pharsight Corporation, build Version 6.4) using nominal doses and sampling times.

Statistics

For the respiratory analysis, data were tabulated within each summary time interval and the arithmetic mean, number of animals, least squares mean and standard error were calculated for each endpoint and treatment. The endpoints of RR, TV, MV, Ti and PIF were analyzed by descriptive statistics and repeated measures analysis of covariance (rANCOVA) (Littell et al., 2006) using the mixed model analysis procedure (STAT System; SAS Institute, Cary, NC). The effect of treatment over week and time during each analysis segment was evaluated using a repeated measures analysis of variance. Factors in the model include gender, treatment, week, time, and all possible interactions with treatment, week, and time. A mixed model approach using SAS was used for the analysis using a Spatial Power covariance structure. The Kenward Roger degrees of freedom approximation was utilized. The effects of treatment, the ‘treatment by week’ interaction, the ‘treatment by time’ interaction, and the ‘treatment by week by time’ interaction were tested using overall F tests at the 0.05 significance level. To determine statistical significance of histopathological data, chi squared tests for multiple comparisons were performed and followed by post hoc pairwise chi squared tests.
RESULTS

In Life Observations

In the head-to-head comparison of reveglucosidase alfa and rhGAA, there were no clinical observations other than the expected reactions to administration of a heterologous protein. Unscheduled mortalities occurred on Day 16 (6 animals given reveglucosidase alfa, 2 animals given rhGAA) and on Day 22 (four animals given reveglucosidase alfa, one animal given rhGAA) following the third and fourth dose and were preceded by observations of lethargy and shallow breathing, suggesting an anaphylactoid-like response. In the studies examining respiratory function, most animals dosed with reveglucosidase alfa exhibited anaphylactoid-like signs such as decreased activity, prostration, low carriage and slow or shallow breathing. Dose- and time-dependent increases in unscheduled mortality occurred during both 4- and 12-week Phases of the study (3/30 and 7/30 for animals given 20 mg/kg reveglucosidase alfa over 4- and 12-weeks, respectively). The cause of mortality was not definitively determined, although likely due to incompletely suppressed anaphylactoid-like reactions potentially due to diphenhydramine’s lack of anti-serotonergic properties. All animals generally gained or maintained body weight normally over the course of the studies and body weight did not differ between group on average more than a gram over the four week phase.

Glycogen Clearance in Muscle Tissue Following administration of Reveglucosidase alfa or rhGAA

In order to evaluate effectiveness of muscle glycogen clearance, a comparison of reveglucosidase alfa with the commercially available form of rhGAA was conducted in a head-to-head
comparison in the Pompe mouse. The dose-response PD modeling results provided estimates of
EC$_{50}$ values for glycogen clearance in five skeletal muscle groups. Both rhGAA and
reveglucosidase alfa were effective in clearing muscle glycogen as evidenced by EC$_{50}$ values
within the dose ranges evaluated (Figure 2 and Table 1). For both compounds, the greatest
clearance was observed in the heart and least in psoas muscle. For reveglucosidase alfa, the
range of EC$_{50}$ values for the five muscle groups was < 3-fold and for rhGAA > 4-fold by dose
(nmol/kg). By comparison reveglucosidase alfa appeared to be more effective than rhGAA in
clearing glycogen in all muscle groups with EC$_{50}$ values based on molar dose levels that were 2.8
to 4.7-fold lower (EC$_{50}$ ratios Table 1). This indicates that on a molar dose basis,
reveglucosidase alfa is more potent than rhGAA at clearing muscle glycogen.

**Respiratory Evaluations**

**PHASE 1, 4-Week Treatment**

Over a 4-week period, improvement in some respiratory parameters were observed in Pompe
mice given 20 mg/kg reveglucosidase alfa, but not in mice given 12 mg/kg reveglucosidase alfa.
These treatment-related changes included 13, 5 and 7% change in TV, MV and PIF relative to
WT during the hypercapnic challenge while vehicle treated Pompe mice showed less
improvement compared to WT (Table 2). PIF (mL/s) under hypercapnic conditions was 11.931 ±
0.772, 8.841 ± 0.772 and 11.209 ± 0.535 in WT, vehicle treated and 20 mg/kg reveglucosidase
alfa treated Pompe mice, respectively. No treatment-related improvements were observed during
the hypercapnic challenge in RR or T$_i$ parameters. Generally, male and female animals
responded similarly during the hypercapnic period and the genders are discussed as combined
data unless otherwise noted.
TV increased in all animals, in response to the hypercapnic challenge (Table 2). In Pompe vehicle control mice, the TV (mL) was 10% lower than the WT mice. In contrast, in Pompe mice given 20 mg/kg reveglucosidase alfa, the TV parameter (mL) increased 13% over WT animals. The overall group effect analysis of TV during the post hypercapnic period indicated that TV (mL) was increased 23% in Pompe mice given 20 mg/kg reveglucosidase alfa. Because TV is the difference between volumes after a normal inhalation and a normal exhalation, treatment related improvement in breathing volume is likely related to increased muscle function. All animal groups mean body weight values remained within 1g over the 4 week period.

MV also increased in all animals in response to the hypercapnic challenge (Table 2). In Pompe vehicle control animals, MV (mL/minute) was 27% lower than WT animals. In Pompe mice given 20 mg/kg reveglucosidase alfa, MV was more similar to WT animals with MV (mL/min) only 5% lower than WT animals. The treatment related improvement in MV is likely due to changes in TV, as RR did not improve with reveglucosidase alfa treatment.

PIF increased in all animals in response to the hypercapnic challenge (Table 2). In Pompe vehicle control animals, PIF (mL/second) was 26% below the WT values. In Pompe animals given 20 mg/kg reveglucosidase alfa, PIF (mL/second) was only 7% lower than WT values that indicated that reveglucosidase alfa treatment increased PIF in response to a hypercapnic challenge (Figure 3). Improvement in PIF was inversely correlated with diaphragm glycogen clearance (Figure 4).

PHASE 2, 12-week treatment
In a subset of animals prior to reveglucosidase alfa treatment, disease progression of respiratory function was measured over 12-weeks prior to the start of the reveglucosidase alfa administration (-12, -8 and -4 weeks, pretreatment). During the hypercapnic period, respiratory function values in Pompe animals progressively decreased in most parameters (TV, MV, PIF, PEF) relative to WT values over time (Supplemental Table 1). Inspiration and expiration time did not change over time. This indicated that disease progression increased over the span of time until treatment initiation. There was no indication that the baseline values changed in these animals due to evaluations prior to drug administration.

Over a 12-week period, improvement in PIF- and PEF-related mean changes from baseline were observed during the hypercapnic challenge in Pompe mice given 20 mg/kg reveglucosidase alfa, but not in Pompe mice given 12 mg/kg reveglucosidase alfa. Increased mean changes from baseline and significant absolute PIF were observed in Pompe mice given 20 mg/kg reveglucosidase alfa during the hypercapnic challenge (10.681±0.374 mL/second, mean change: 0.517 mL/second, change from baseline: 5%) and post-hypercapnic recovery (4.937±0.203 mL/second, mean change: 0.303 mL/second, change from baseline: 6.5%) compared to vehicle treated Pompe mice (9.068±0.349 mL/second, mean change: -0.688 mL/second, and 4.203±0.187, mean change: -.0.132, for hypercapnic and recovery, respectively) after 12-weeks (Supplemental Table 2). Overall PIF decreased over time in the untreated Pompe mouse over the hypercapnic and recovery period. Improvement after 12-weeks was most evident in PEF during the hypercapnic challenge (12.052±0.392 mL/second, mean change: 1.223 mL/second, change from baseline: 11%) as well as the post-hypercapnic recovery period (4.598±0.152 mL/second, mean change: 1.223 mL/second, change from baseline: 10.5%) in Pompe mice given
20 mg/kg reveglucosidase alfa (Supplemental Table 3) with no differences were observed compared to WT animals. During the 12-weeks, disease progression was evident in the untreated Pompe mice. In Pompe mice given 20 mg/kg reveglucosidase alfa for 12 weeks, general stabilization in respiratory function, rather than a disease related decline, was observed during the hypercapnic and recovery phases, as evidenced by PIF (Figure 5) and PEF (Figure 6), increases in MV and TV without observations of compensation in RR, T_i, or T_e. Vehicle treated Pompe animals generally showed no improvement over baseline in PIF and PEF and respiratory function generally did not improve over time. No treatment-related improvements were observed during the hypercapnic challenge in RR, T_e or T_i parameters (data not shown). Generally, male and female animals responded similarly during the hypercapnic period.

**Macroscopic and Microscopic Pathology**

No reveglucosidase alfa -related macroscopic findings were observed in the Phase 2, 12-week study. Disease-related findings were observed in the intercostal muscle, brain, spinal cord, and dorsal root ganglia of the Pompe vehicle treated animals (Table 3). Findings in the intercostal muscle included vacuolation, degeneration and increased glycogen compared to concurrent WT animals. Additional disease-related findings included neuronal vacuolation (brain, spinal cord, dorsal root ganglia), increased neuronal glycogen content (brain, spinal cord, dorsal root ganglia and Schwann cells of the phrenic nerve), decreased myelin content (corpus callosum of the brain), axonal degeneration in the dorsal tracts of the spinal cord (largely afferrent/sensory tracts) and degeneration of axons in dorsal and ventral spinal nerve roots compared to concurrent WT control animals. In the Pompe animals treated with 20 mg/kg reveglucosidase alfa, decreased muscle degeneration and glycogen content was observed in the intercostal muscle. Additionally,
a relative decrease of neuronal vacuolation and glycogen accumulation in the brain and spinal cord and a possible decreased effect on myelin in the corpus callosum was observed compared to the vehicle treated Pompe animals indicating possible beneficial exposure to reveglucosidase alfa. There was no discernible treatment related effect on the glycogen in the dorsal root ganglia or in the Schwann cells of the phrenic nerve potentially due to method of fixation (Lynch et al, 2005).

Glycogen Tissue Analysis

PHASE 1, 4-Week Treatment

Reveglucosidase alfa dose related reductions in glycogen content in heart, diaphragm, quadriceps, gastrocnemius, and psoas muscles as compared to vehicle treated Pompe animals (Supplemental Tables 4 and 5). In animals given 20 mg/kg reveglucosidase alfa, there was an overall decrease in glycogen in quadriceps, gastrocnemius, diaphragm, heart ventricle, and psoas muscles reaching significance compared to vehicle treated Pompe animals (Supplemental Table 5); Only glycogen storage in the heart decreased to WT levels at the dose levels tested.

PHASE 2, 12-Week Treatment

In the vehicle-treated Pompe mouse, glycogen storage was significantly above wild type levels in all muscles analyzed (diaphragm, abdominals, heart, quadriceps, gastrocnemius and psoas). Reveglucosidase alfa treatment significantly reduced glycogen in all muscles compared to Pompe controls (Table 4) although glycogen levels did not decrease to WT control levels following 12-weeks of reveglucosidase alfa treatment. Compared to vehicle-treated Pompe controls, animals given 20 mg/kg reveglucosidase alfa achieved significant decreases in
glycogen content in the left ventricle, diaphragm, soleus, quadriceps, gastrocnemius, and left psoas after 12-weeks of treatment (for each, \( p < 0.05 \)). Based on ED\(_{50}\) calculations, a 50% reduction in muscle glycogen content was achieved at < 20 mg/kg in all tissues at all dose levels (Table 5). Reveglucosidase alfa was most effective in heart, followed, in order of increasing EC\(_{50}\), by soleus, gastrocnemius, quadriceps, diaphragm, abdomen and psoas muscles. Relative glycogen accumulation decreased in the intercostal muscles of animals given 20 mg/kg reveglucosidase alfa relative to vehicle treated animals (Table 3). Additionally, disease-related vacuolation and degeneration in myofibers and nervous system, which are consequences of glycogen accumulation in Pompe mice, were reduced in the animals given 20 mg/kg reveglucosidase alfa compared to the vehicle treated Pompe animals. These relative decreases in glycogen indicated that reveglucosidase alfa exposure occurred in these tissues, though absolute microscopic analysis was not possible due to use of method of fixation and PAS staining (Lynch et al, 2005).

**Pharmacokinetics (PK)**

Single-dose PK of reveglucosidase alfa in WT mice was used to determine relative exposure estimates in the Pompe mouse model. Overall, exposure to reveglucosidase alfa increased with increasing dose and reveglucosidase alfa plasma concentrations rapidly declined following IV administration. The plasma half -life of elimination was rapid and calculated to be < 0.5 hours in both genders. The plasma clearance rate was categorized as low (< 1/10 hepatic plasma blood flow rate for a 20-25 g mouse referenced to 3000 mL/h/kg; Kwon ed, 2001) with clearance rates higher in females (131 mL/h/kg) than males (104 mg/h/kg). The volume of distribution was categorized as low (< total body water) with the volume of distribution slightly higher than
plasma volume (49 mL/kg) and higher in females (69.2 mL/kg) than males (53.2 mL/kg).

Exposure was dose proportional for \( C_{\text{max}} \) in both genders. Exposure measured by \( \text{AUC}_{0-\text{inf}} \) increased in a greater than dose proportional manner and at a < 2:1 ratio. Male mice had higher exposure by \( \text{AUC}_{0-t} \) than the females at the low (+21%) and mid (+56%) doses with essentially the same exposure at the 20 mg/kg dose (male +8%).

In order to translate the efficacy observed in the mouse with human exposure, a dose–exposure analysis was conducted. Both body surface area based methodology and linear extrapolation of actual revenglucosidase alfa dose-exposure data in the mouse (4 to 20 mg/kg) were used to determine a human to mouse dose equivalence (Food and Drug Administration, 2005). A linear extrapolation of a plot of mouse dose-exposure data out to exposure levels observed in human given 20 mg/kg revenglucosidase alfa (\( \text{AUC}_{0-\text{inf}} =1,807,050 \text{ ng*h/mL}; \) Byrne et al, in preparation) indicates that an equivalent dose in mouse would be at 184 mg/kg. Body surface area calculations indicated a 246 mg/kg dose in mouse to be equivalent to a maximal 20 mg/kg dose in human. Based on these types of dose-exposure comparisons, the respiratory correction observed in Pompe mouse given 20 mg/kg dose was achieved at an approximate 9- to 12-fold lower revenglucosidase alfa exposure than observed in Pompe patients receiving maximal dosing at 20 mg/kg (Byrne et al, in preparation).
DISCUSSION

Pompe disease is defined by a spectrum of mutations in the acid alpha-glucosidase gene leading to the accumulation of lysosomal glycogen in skeletal, cardiac and smooth muscles, the CNS and other organs (Thurberg 2006, Sidman RL et al., 2008; Fuller DD et al., 2013). In patients with LOPD, muscle wasting and complications in pulmonary function are the hallmark of disease phenotype (Smith et al., 2013). In LOPD patients, respiratory-related glycogen accumulation is observed early along with observations of glycogen accumulation in cardiac, vascular smooth muscle, skeletal muscle and organs with smooth muscle (bladder, intestine, esophagus) that match to the clinical correlates of respiratory dysfunction with occasional gastrointestinal dysfunction and urinary incontinence (Rabin et al, 1998, Hobson-Webb et al, 2012). Long-term use of rhGAA ERT can modulate respiratory dysfunction by decreasing the number of ventilator hours, with stabilization of FVC generally observed in patients relative to untreated controls (van der Ploeg et al, 2010, Vianello et al, 2013). Improvements in maximal expiratory pressure (MEP) and maximal inspiratory pressure (MIP) occurred in the earlier timeframe (12 and 26 weeks, respectively). Similarly, in the Pompe mouse respiratory study, treatment related improvements in PEF and PIF were observed during the first half of the study (Figure 5 and 6). Additionally, treatment related improvements in hypercapnic PEF, as measured by absolute mean change, were greater than PIF absolute mean change over the course of this study.

The Pompe mouse demonstrates biochemical and pathological changes similar to affected patients (Raben N et al., 1998) and recapitulates the respiratory features of the disease. The progressive muscle weakness, neural changes and pulmonary insufficiency observed with reduced mobility and muscle wasting are correlated with increasing lysosomal glycogen.
accumulation (Sidman RL et al., 2008, DeRuisseau et al., 2009). In these studies, 4-month old Pompe mice were used to ensure high relative levels of glycogen storage in the skeletal and diaphragmatic muscles and neurons (Raben N et al., 1998), prior to significant loss of muscle strength at 8 months (Sidman et al., 2008). This age of mouse is most similar to a patient with LOPD after a late diagnosis. In the clinic, early diagnosis and treatment of LOPD patients is thought to increase treatment benefit with enzyme replacement therapy (Chien et al., 2013), thus making the ability to achieve significant treatment-related change more difficult with delayed initiation of treatment.

Reveglucosidase alfa confers improved enzyme uptake into the lysosome because of the GILT tag that utilizes the IGF2R region of the CI-MPR rather than depending on the glycosylation pattern of the GAA protein and the M6P region of the CI-MPR for uptake to the lysosome (Maga JA et al., 2013). To test the validity of increased uptake into the lysosome via the IGF2R mechanism, a 5-week dose response comparison of reveglucosidase alfa and rhGAA revealed that targeting the IGF2R region as an uptake mechanism over the M6P region resulted in increased glycogen clearance. Relative EC\textsubscript{50} ratios of rhGAA/ reveglucosidase alfa enabled a comparison on a molar basis and demonstrated that less reveglucosidase alfa is needed to effectively clear glycogen in the diaphragm (Figure 2), heart, quadriceps, psoas and soleus muscles (Table 1). In the 12-week respiratory study with longer duration of reveglucosidase alfa treatment, the EC\textsubscript{50} values for glycogen clearance were lower than that for the muscles in the rhGAA/ reveglucosidase alfa comparison (Tables 1) where improvement in respiratory outcomes for PEF and PIF was observed.
Reveglucosidase alfa, with its increased cellular uptake, was an ideal agent to study the therapeutic impact of glycogen reduction on pulmonary function. After short-term treatment with 20 mg/kg reveglucosidase alfa for 4 weeks, improvement in PIF, MV and TV under hypercapnic and recovery conditions were observed. After 12 weeks of 20 mg/kg reveglucosidase alfa treatment, PEF improvement was greater than PIF during hypercapnic and recovery conditions compared to vehicle treated Pompe mouse. Both PIF and PEF showed a durable response after 4-6 weeks in this study (Figures 5 and 6). PIF and PEF changes were generally significant over time and PEF improved into the range of normal WT animals during the normoxic post hypercapnic recovery. During hypercapnic evaluations, PIF improved compared to vehicle-treated Pompe animals but not into the normal WT range, indicating the increased muscle activity and neuronal control needed to compensate for an increased CO₂ level were not completely achieved. This response was likely mediated by the extent of disease progression in these animals prior to start of treatment. However, the response to the hypercapnia should be similar to respiratory gas exchange and acid-base status observed in a patient with LOPD (Mellies and Lofaso, 2008). Overall, respiratory improvements in PIF and PEF was supported by decreased glycogen levels in diaphragm, abdominal, and intercostal muscles at the end of 12 weeks when Pompe mice were almost 7-months old. The improvement in PIF and PEF is likely an early indicator of stabilized respiratory function. This functional change is promising considering the age of the animals used in this study. A linear extrapolation of mouse AUC₀-inf exposure data to reach exposure levels observed in humans given 20 mg/kg reveglucosidase alfa (1,807,050 ng·h/mL) indicates that an equivalent dose in mouse would be approximately 166 mg/kg. Based on these dose-exposure extrapolations, the respiratory
correction observed in Pompe mouse given 20 mg/kg of reveglucosidase alfa was achieved at an approximate 8-fold lower reveglucosidase alfa exposure than the on-going clinical trial in Pompe patients (Byrne et al, in preparation) though the physiologic translation between species is unknown.

Disease related histopathology was used as a surrogate marker to assess relative reveglucosidase alfa exposure in tissues where use of a direct glycogen assay was difficult (intercostal muscle layers and CNS). Reveglucosidase alfa related decreases in glycogen storage were observed in the intercostal muscle similar to other respiratory muscles. Treatment with reveglucosidase alfa reduced the glycogen accumulation in some brain tissues as scored using light microscopy indicating the possible exposure to reveglucosidase alfa in certain areas of the CNS. Glycogen accumulation in the CNS has been more accurately analyzed in six-month old and older Pompe mice using an enhanced staining method (fluorogold and PAS) (DeRuisseau et al, 2009). Although glycogen accumulation has been documented in this mouse model in motor nerves, particularly at the level of the spinal cord (Sidman RL et al, 2008, Turner et al, 2016), the relative disease pathology of this particular Pompe mouse colony may preclude definitive evidence of glycogen changes within the nervous system. Functional neural deficits including decreased inspiratory burst amplitude have been noted in the phrenic nerve (Sidman RL et al., 2008; DeRuisseau LR et al., 2009) along with histological changes of the neuromuscular junction (NMJ) along with contractile dysfunction in the diaphragm (Falk et al 2013; Falk et al 2015) ; however, the extent of glycogen accumulation required to effect functional change in these tissues is not known. Stage of disease progression can influence the ability of a therapeutic to enact functional and anatomical change. Heterogeneity was observed in human autopsy
reports where glycogen granules were reported in Schwann cell bodies, neurons, spinal cord and peripheral nerves in some but not all of the evaluations (Hobson-Webb et al, 2013). Gene therapy treatment in older Pompe mice does not modify the NMJ, area of motor endplate, area occupied by acetylcholine receptors nor affect force restoration despite clearance of glycogen storage in the muscle (Todd 2015).

In a comparison of glycogen clearance with reveglucosidase alfa and rhGAA, reveglucosidase alfa was more effective than rhGAA with 2.8 to 4.7 lower EC50 values in five muscles, likely due to increased cellular uptake. Improvement in respiratory function (PEF and PIF) during hypercapnic and recovery periods, was quantitatively observed in mice at extrapolated reveglucosidase alfa exposure levels significantly lower than those achieved clinically at the same dose level. Decreased disease related microscopic findings indicate that ambulatory and respiratory muscle along with areas of the brain and spinal cord were exposed to reveglucosidase alfa suggesting better drug distribution resulting in a potential overall effect on respiratory function. Decreased glycogen storage in the respiratory muscles, (diaphragm, abdominals and intercostal muscles) supports improvements and stabilization of PIF and PEF values over time in the Pompe mouse. These improvements in PIF and PEF during hypercapnia and post-hypercapnic recovery periods suggest increased muscle strength was correlated to decreased glycogen storage. Relative mean changes from baseline in PEF were greater than PIF in reveglucosidase alfa treated Pompe mice. PIF and PEF generally declined or did not improve in the untreated Pompe mice, respectively over 12-weeks. Despite these complexities, improvement in respiratory function (PEF and PIF) during hypercapnic and recovery periods, was quantitatively observed at extrapolated reveglucosidase alfa exposure levels significantly
lower than those achieved clinically at the same dose level. Therefore, correction of the respiratory manifestations of Pompe disease with enzyme replacement therapy may be dependent on treatment duration, disease progression or disease severity. Improvement of murine PIF and PEF were similar in magnitude to increases in MIP and MEP observed in late onset Pompe patients treated with reveglucosidase alfa (Byrne et al, in preparation).
ACKNOWLEDGEMENT

The authors acknowledge the editorial assistance of Dr. Carl S. Hornfeldt, Randall Lee and Dr. Carley Corado during the preparation of this manuscript; Ruben Martinez, Lucy Crockett, Ruby Cheung (BioMarin Pharmaceutical Inc., Novato, CA) and Michael Paton (KCAS Bioanalytical Services, Shawnee, KS) for their bioanalytical support.
AUTHORSHIP CONTRIBUTION

Participated in research design: Peng, Dalton, Butt, Kennedy, Haroldsen, O’Neill and Tsuruda

Conducted experiments: Dalton and Butt

Contributed new reagents or analytical tools: Tracy, Cahayag and Zoog

Performed data analysis: Peng, Dalton, Butt, Kennedy, Zoog and Tsuruda

Wrote or contributed to the writing of the manuscript: Peng, Tracy, Haroldsen, O’Neill and Tsuruda
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Footnotes

This work was supported by BioMarin Pharmaceutical, Inc., Novato, CA.
FIGURES

Figure 1. Comparison of Reveglucosidase alfa and Recombinant Human Acid alpha(α)-Glucosidase (rhGAA)

Reveglucosidase alfa contains a variant of the human insulin-like growth factor 2 (IGF2) (residues 1 and 7-67; black) linked by a three amino acid spacer (GAP; red) to residues 70-952 of hGAA (blue) that targets the IFG2 receptor region of the cation independent mannose-6-phosphate receptor (CI-M6PR) and is not dependent on glycosylation for cellular uptake into the lysosome. The hGAA residues in Reveglucosidase alfa are identical to the corresponding residues in alglucosidase alfa as indicated by the blue.

Figure 2. Glycogen Clearance Comparison by Dose Between Recombinant Human Acid alpha(α)-Glucosidase (rhGAA) and Reveglucosidase alfa in Pompe Mouse Diaphragm

Glycogen changes in diaphragm of mice given a single IV dose of 12, 20 or 60 mg/kg rhGAA and 4, 12 or 60 mg/kg reveglucosidase alfa after 4 weeks, post dose. N= 9/group

Figure 3. Treatment with Reveglucosidase alfa Improves Peak Inspiratory Flow, Phase 1

Mean (SD) changes in PIF, as measured by a plethysmography chamber, were compared in reveglucosidase alfa -treated Pompe mice (20 mg/kg, green line), vehicle-dosed Pompe mice (red line), and vehicle dosed WT mice (blue line). Animals were subjected to hypercapnic conditions for 15 min after an average acclimation period of 50 minutes. For each point, N=5-10 animals. * denotes p<0.05 comparing vehicle dosed WT and Pompe mice.

Figure 4. Improvement in Peak Inspiratory Flow is Inversely Correlated with Glycogen Clearance in Diaphragm Muscles, Phase 1
Mean (SD) improvement in PIF (orange) in reveglucosidase alfa-treated Pompe mice was associated with a reduction of glycogen levels (blue) in diaphragm muscles. PIF data was obtained from t=10 minutes post-initiation of hypercapnic conditions. The reduction in glycogen, assessed at 4-weeks, was associated with an increase in PIF during hypercapnia challenge ($r = -0.58$, $p=0.01$). For each point, $N=5-10$ animals.

**Figure 5. Reveglucosidase alfa Treatment in Pompe Mice Maintained Improvement in Peak Inspiratory Flow over 12-Weeks**

Weekly treatment with 20 mg/kg reveglucosidase alfa (green) in Pompe mice resulted in increased PIF over vehicle treated (red) Pompe mice. Improvement in PIF was observed in A. Normoxic (pre-hypercapnia), B. hypercapnic, and C. normoxic (recovery) conditions. For each time point N=9-10 for WT group, N=22-30 for Pompe mouse groups; Shaded area denotes absolute values of wild type vehicle treated WT mice. Mean ± Standard Error. *denotes p<0.05 vs Pompe mice given vehicle.

**Figure 6. Reveglucosidase alfa Treatment in Pompe Mice Maintained Improvement in Peak Expiratory Flow (PEF) over 12-Weeks**

Weekly treatment with 20 mg/kg reveglucosidase alfa (green) in Pompe mice resulted in increased PEF over vehicle treated (red) Pompe mice. Improvement in PEF was observed in A. Normoxic (pre-hypercapnia), B. hypercapnic, and C. normoxic (recovery) conditions. For each time point N=9-10 for WT group, N=22-30 for Pompe mouse groups; Shaded area denotes absolute range values of vehicle treated WT mice. Mean ± Standard Error. *denotes p<0.05 vs Pompe mice given vehicle.
TABLES

Table 1. Glycogen Clearance Comparison by Muscle Type between Reveglucosidase alfa and rhGAA in Pompe Mice

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>rhGAA $EC_{50}$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reveglucosidase alfa</th>
<th>$EC_{50}$&lt;sup&gt;a&lt;/sup&gt; Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Ratios&lt;sup&gt;reveglucosidase alfa&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
<td>rhGAA/</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>13.2 (0.893)</td>
<td>5.00 (0.298)</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>32.2 (4.64)</td>
<td>12.1 (1.96)</td>
<td>2.88</td>
<td></td>
</tr>
<tr>
<td>Quadriceps</td>
<td>25.2 (4.59)</td>
<td>9.70 (1.12)</td>
<td>2.81</td>
<td></td>
</tr>
<tr>
<td>Psoas</td>
<td>54.6 (10.7)</td>
<td>12.4 (2.82)</td>
<td>4.77</td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>27.8 (3.47)</td>
<td>9.36 (1.48)</td>
<td>3.22</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> mean (SE)

<sup>b</sup> to account for differences in molecular weight, a correction factor of 1.0825 was used to convert rhGAA doses in mg/kg to equivalent doses of reveglucosidase alfa in mg/kg for EC50 ratio comparison. (Molecular weight: rhGAA = 109 kDA, reveglucosidase alfa = 118 kDA)
Table 2: Phase 1, Percent Changes in Respiratory Parameters after 4-Weeks in Pompe mouse Given Vehicle or Reveglucosidase alfa

<table>
<thead>
<tr>
<th>Respiratory Parameter</th>
<th>% Change Relative to Baseline</th>
<th>% Change Relative to WT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (N=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pompe Vehicle (N=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pompe 20 mg/kg Reveglucosidase alfa (N=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV(^a)</td>
<td>↑365%</td>
<td>↓10%</td>
</tr>
<tr>
<td>MV(^a)</td>
<td>↑394%</td>
<td>↓27%</td>
</tr>
<tr>
<td>PIF</td>
<td>↑260%</td>
<td>↓26%</td>
</tr>
<tr>
<td>RR</td>
<td>↓24%</td>
<td>↓13%</td>
</tr>
<tr>
<td>Ti</td>
<td>↑31%</td>
<td>↓19%</td>
</tr>
</tbody>
</table>

TV: tidal volume where TV = MV*RR; MV: minute volume; PIF: peak inspiratory flow; RR: respiratory rate; Ti: inspiratory time

\(^a\) there were no notable differences in body weight among the animals over the course of the study, therefore these parameters were not normalized for body weight.
### Table 3: Pompe Disease-Related Microscopic Findings, Phase 2, 12-Weeks

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Diagnosis</th>
<th>Group 1 Wild Type 0 mg/kg</th>
<th>Group 2 Pompe, Vehicle 0 mg/kg</th>
<th>Group 3 Pompe, Reveglucosidase alfa 20 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>INC</td>
<td>SEV</td>
<td>INC</td>
</tr>
<tr>
<td>Brain, Cerebral Cortex</td>
<td>Neurons, Glycogen Accumulation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain, Midbrain</td>
<td>Neurons, Glycogen Accumulation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain, Cerebellum</td>
<td>Neurons, Glycogen Accumulation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brain, Pons Region/Medulla Oblongata</td>
<td>Neurons, Glycogen Accumulation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
### Spinal Cord, Lumbar

<table>
<thead>
<tr>
<th>Neurons, Glycogen Accumulation</th>
<th>0/5</th>
<th>0.00</th>
<th>5/5(^a)</th>
<th>5.00</th>
<th>5/5(^a)</th>
<th>2.60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5(^a)</td>
<td>5.00</td>
<td>5/5(^a)</td>
<td>2.40</td>
</tr>
<tr>
<td>Dorsal Spinal Nerve Roots, Degeneration</td>
<td>0/5</td>
<td>0.00</td>
<td>4/5(^a)</td>
<td>1.80</td>
<td>4/5(^a)</td>
<td>1.40</td>
</tr>
<tr>
<td>Ventral Spinal Nerve Roots, Degeneration</td>
<td>0/5</td>
<td>0.00</td>
<td>4/5(^a)</td>
<td>2.40</td>
<td>5/5(^a)</td>
<td>2.00</td>
</tr>
</tbody>
</table>

### Dorsal Root Ganglia

<table>
<thead>
<tr>
<th>Neurons, Glycogen Accumulation</th>
<th>0/5</th>
<th>0.00</th>
<th>5/5(^a)</th>
<th>5.00</th>
<th>5/5(^a)</th>
<th>4.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurons, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5(^a)</td>
<td>4.60</td>
<td>5/5(^a)</td>
<td>3.60</td>
</tr>
<tr>
<td>Nerve, Axonal Degeneration</td>
<td>0/5</td>
<td>0.00</td>
<td>1/5</td>
<td>0.40</td>
<td>0/5</td>
<td>0.00</td>
</tr>
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</table>

### Intercostal Muscle

<table>
<thead>
<tr>
<th>Muscle, Increased PAS Staining</th>
<th>0/5</th>
<th>0.00</th>
<th>5/5(^a)</th>
<th>3.60</th>
<th>5/5(^a)</th>
<th>2.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibers, Vacuolation</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5(^a)</td>
<td>3.60</td>
<td>5/5(^a)</td>
<td>1.80</td>
</tr>
<tr>
<td>Myofibers, Degeneration</td>
<td>0/5</td>
<td>0.00</td>
<td>5/5(^a)</td>
<td>3.20</td>
<td>5/5(^a)</td>
<td>1.80</td>
</tr>
<tr>
<td>Intercostal Nerves, Axonal Degeneration</td>
<td>0/5</td>
<td>0.00</td>
<td>2/5</td>
<td>0.40</td>
<td>0/5</td>
<td>0.00</td>
</tr>
</tbody>
</table>

INC=Incidence; the number of animals with a particular finding / total number of animals in the group; SEV=Average Severity; the sum of individual severity grades divided by the number of animals in the group.

\(a\) significantly different from 0 mg/kg/dose Vehicle (WT); \(p<0.05\)

\(b\) significantly different from 0 mg/kg/dose Vehicle (GAA); \(p<0.05\)

grading scheme: Grade 1: barely exceeds normal limits; not readily apparent at evaluation; Grade 2: more apparent than Grade 1 but unlikely to cause structural/functional impairment; Grade 3: readily apparent in the tissue but of limited severity; Grade 4: prominent change expected

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to have some effect on structure and/or function; Grade 5: Present at or near a maximum severity; expected to have a pronounced effect on structure/function
Table 4: Muscle Tissue Glycogen Concentration in Animals given 20 mg/kg Reveglucosidase alfa for 12-Weeks

<table>
<thead>
<tr>
<th></th>
<th>WT/Vehicle, N=9</th>
<th>Pompe/Vehicle, N=28</th>
<th>Pompe/20 mg/kg, N=22</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diaphragm, mg/g, mean (SD)</strong></td>
<td>0.56 (0.62)</td>
<td>12.6 (2.73)</td>
<td>3.20 (1.78)</td>
</tr>
<tr>
<td><strong>Abdominals, mg/g, mean (SD)</strong></td>
<td>0.05 (0.19)</td>
<td>8.34 (2.36)</td>
<td>2.64 (2.18)</td>
</tr>
<tr>
<td><strong>Heart, left ventricle, mg/g, mean (SD)</strong></td>
<td>0.01 (0.08)</td>
<td>21.4 (3.68)</td>
<td>1.31 (1.34)</td>
</tr>
<tr>
<td><strong>Left Quadriceps, mg/g, mean (SD)</strong></td>
<td>0.00 (0.14)</td>
<td>7.25 (0.94)</td>
<td>1.37 (1.54)</td>
</tr>
<tr>
<td><strong>Left Gastrocnemius, mg/g, mean (SD)</strong></td>
<td>0.16 (0.06)</td>
<td>8.29 (0.94)</td>
<td>1.54 (1.74)</td>
</tr>
<tr>
<td><strong>Left Psoas, mg/g, mg/g, mean (SD)</strong></td>
<td>0.06 (0.11)</td>
<td>8.90 (1.91)</td>
<td>4.56 (2.39)</td>
</tr>
</tbody>
</table>

*a significantly different from WT/Vehicle, (p<0.05); b significantly different from WT/Vehicle (WT), (p<0.01); c significantly different from Pompe/Vehicle, (p<0.05); d significantly different from Pompe/Vehicle (Pompe), (p<0.01).
Table 5. Glycogen clearance by Muscle Type following 12 weeks of Treatment with 20 mg/kg Reveglucosidase alfa in Pompe Mice

<table>
<thead>
<tr>
<th>Muscle Type</th>
<th>EC$_{50}$ (mg/kg)$^a$</th>
<th>Male</th>
<th>Female</th>
<th>M + F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.72 (0.324)</td>
<td>1.94 (0.320)</td>
<td>1.81 (0.223)</td>
<td></td>
</tr>
<tr>
<td>Diaphragm</td>
<td>6.06 (0.886)</td>
<td>7.44 (0.814)</td>
<td>6.98 (0.632)</td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>8.62 (1.67)</td>
<td>9.72 (1.72)</td>
<td>9.06 (1.24)</td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>3.29 (0.515)</td>
<td>3.07 (0.503)</td>
<td>3.15 (0.335)</td>
<td></td>
</tr>
<tr>
<td>Quadriceps</td>
<td>4.02 (0.778)</td>
<td>4.66 (0.641)</td>
<td>4.47 (0.558)</td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>3.11 (0.492)</td>
<td>5.29 (0.673)</td>
<td>4.39 (0.443)</td>
<td></td>
</tr>
<tr>
<td>Psoas</td>
<td>7.02 (1.45)</td>
<td>16.7 (3.30)</td>
<td>10.5 (1.56)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Mean (SE)
FIGURE 2

Glycogen (mg/gm) vs. Dose (mg/kg)
FIGURE 4

Glycogen (mg/g) vs. Reveglucosidase alfa (mg/kg)

Peak Inspiratory Flow (mL/s)

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FIGURE 5

A. Normoxic (Pre-hypercapnia)

B. Hypercapnic

C. Normoxic (Recovery)