APPLICATION NUMBER:
125141/0

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)
<table>
<thead>
<tr>
<th><strong>BLA</strong></th>
<th>125141</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Submission Date</strong></td>
<td>July 29, 2005</td>
</tr>
<tr>
<td><strong>Action Due Date</strong></td>
<td>April 28, 2006</td>
</tr>
<tr>
<td><strong>Proposed Trade Name</strong></td>
<td>Myozyme</td>
</tr>
<tr>
<td><strong>Generic Name</strong></td>
<td>a-glucosidase alfa; recombinant human acid alpha-glucosidase (rhGAA)</td>
</tr>
<tr>
<td><strong>Route of Administration</strong></td>
<td>Intravenous</td>
</tr>
<tr>
<td><strong>Formulation, Strength</strong></td>
<td>Lyophilized cake or powder for reconstitution; each vial contains a total extractable volume of 10 mL at 5.0 mg/mL a-glucosidase alfa following reconstitution</td>
</tr>
<tr>
<td><strong>Sponsor</strong></td>
<td>Genzyme Corp.</td>
</tr>
<tr>
<td><strong>Relevant IND(s)</strong></td>
<td>BB-IND 10780</td>
</tr>
<tr>
<td><strong>Submission Type</strong></td>
<td>NME, Priority</td>
</tr>
<tr>
<td><strong>Proposed Indication</strong></td>
<td>/</td>
</tr>
<tr>
<td><strong>Dose and Dosing Regimen</strong></td>
<td>20 mg/kg administered every 2 weeks as an approximate 4-hour intravenous infusion</td>
</tr>
<tr>
<td><strong>Division / Team</strong></td>
<td>Division of Clinical Pharmacology 5 / Biologics Team</td>
</tr>
<tr>
<td><strong>Reviewer</strong></td>
<td>Anil Rajpal</td>
</tr>
<tr>
<td><strong>Team Leader</strong></td>
<td>Hong Zhao</td>
</tr>
<tr>
<td><strong>Division Director (Acting)</strong></td>
<td>Shiew Mei Huang</td>
</tr>
</tbody>
</table>

Appears This Way
On Original
Table of Contents

Table of Contents................................................................. 2
  Table of Tables......................................................................... 2
  Table of Figures........................................................................ 3
1. Executive Summary.................................................................... 3
  1.1 Recommendation................................................................... 3
  1.2 Phase IV Commitments......................................................... 3
  1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings........................................................................ 3
Reviewer, Team Leader, and Division Director Signatures...................... 7
2. Question-Based Review (QBR)...................................................... 8
  2.1 General Attributes.............................................................. 8
  2.2 General Clinical Pharmacology............................................. 9
  2.3 Intrinsic Factors.................................................................... 16
  2.4 Extrinsic Factors................................................................... 22
  2.5 General Biopharmaceutics..................................................... 23
  2.6 Analytical Section............................................................... 26
3. Detailed Labeling Recommendations........................................... 28
4. Appendices................................................................................ 30
  4.1 Proposed Labeling (Original and Annotated)............................. 30
  4.2 Individual Study Reviews...................................................... 57
    4.2.1 Product Development Rationale........................................ 57
    4.2.2 Clinical Pharmacology and Pharmacokinetics Program........... 58
    4.2.3 Pharmacokinetics.......................................................... 59
      Single and Multiple Dose PK.................................................. 59
      Pharmacokinetics in Special Populations................................. 65
      Drug Metabolism and In vitro Drug-Drug Interaction Studies........ 65
      Drug-Drug Interaction Studies.............................................. 65
    4.2.4 Pharmacodynamics........................................................ 65
    4.2.5 Animal Studies.............................................................. 68
    4.2.6 Exposure-Response........................................................ 72
    4.2.7 Dose-Finding Rationale.................................................... 72
    4.2.8 Immunogenicity.............................................................. 73
    4.2.9 General Biopharmaceutics............................................... 78
    4.2.10 Analytical Methods........................................................ 83
  4.3 Consult Review (Pharmacogenomics)........................................ 84
  4.4 Attendance at Required Office Level OCPB Briefing.................... 86

Table of Tables
Table 1. Effect of 20 and 40 mg/kg OQW Alglucosidase Alfa on Selected Clinical Response Measures (Study 1602)...... 11
Table 2. Change from Baseline to Week 26 in LVMII in Each Alglucosidase Alfa Dose Group (Study 1602).................... 11
Table 3. VF Survival vs Ab Titer (PK evaluable subset of Study 1602; n=11)......................................................... 14
Table 4. Sustained IARs vs Ab Titer (Studies 1602 and 1702).............................................................................. 14
Table 5. Ratio of Mean PK Parameters After Single 40 mg/kg Dose to After Single 20 mg/kg Dose (Study 1602) ...... 14
Table 6. Ratio of Mean PK Parameters After 7th Dose to After 1st Dose (Study 1602 and Study 1702)..................... 14
Table 7. Single-dose PK Parameters in Study 1602 and Study 1702 (Mean ± SD)..................................................... 15
Table 8. Multiple-dose PK Parameters in Study 1602 and Study 1702 (Mean ± SD)............................................... 16
Table 9. Ratio of Mean PK Parameters in Study 1702 to Study 1602................................................................. 17
Table 10. Invasive Ventilator-Free Survival and Peak Antibody Titers (Study 1602)............................................. 22
Table 11. Ratio (AUC0-∞ of 2000 L Scale Lots) / (AUC0-∞ of 160 L Scale Lot)......................................................... 24
Table 12. Liver: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot.............................................. 25
Table 13. Quadriceps: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot...................................... 25
1. Executive Summary

1.1 Recommendation

The results of clinical pharmacology and pharmacokinetics studies support the approval of the 160-L scale clinical trial drug product. Because the to-be-marketed 2000-L scale drug product is not pharmacokinetically comparable to the 160-L scale clinical trial product, and the potency assay for alglucosidase alfa is not qualified, clinical efficacy and safety of the 2000-L scale product should be demonstrated to support its approval.

1.2 Phase IV Commitments

There are no Phase IV commitments requested from the Clinical Pharmacology and Biopharmaceutics perspective.

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Mechanism of Action: Pompe disease is an inherited, progressive muscle disease resulting from a deficiency of the lysosomal enzyme acid α-glucosidase (GAA). Deficiency of GAA results in the accumulation of organelle-bound (lysosomal) and extra lysosomal glycogen. Alglucosidase alfa degrades glycogen by catalyzing the hydrolysis of α-1,4- and α-1,6- glycosidic linkages of lysosomal glycogen. Glycogen accumulation in Pompe disease occurs in various tissues, particularly cardiac, respiratory and skeletal muscle, leading to the development of cardiomyopathy and progressive muscle weakness, including impairment of respiratory function. Alglucosidase alfa is intended to provide an exogenous source of GAA for patients with Pompe disease. Nonclinical evaluation with in vivo models of Pompe disease has shown that alglucosidase alfa is able to reverse biochemical and histopathological manifestations of the disease.

Rationale for Dose Selection: Alglucosidase alfa was found to deplete tissue glycogen in GAA knockout mice in a dose-dependent fashion in 3 nonclinical studies, which included weekly dosing at 1, 5, 20, 60, and 100 mg/kg. Higher doses of alglucosidase alfa removed a greater proportion of stored glycogen in a wide variety of tissues, particularly from the heart. Every other weekly (QOW) dosing with alglucosidase alfa appears to be at least as effective as weekly dosing based on two nonclinical depletion/re-accumulation studies conducted in GAA knockout mice using a dose of 100 mg/kg. The time-course of re-accumulation suggested that dosing at 2-week intervals will coincide with the lowest point of glycogen levels in heart and skeletal muscles, and thus provide the greatest depletion of the substrate. Clinical data from the pivotal trial indicated that the 20 mg/kg QOW and 40 mg/kg QOW doses were associated with similar efficacy, but the 20 mg/kg group had a more favorable
safety profile than the 40 mg/kg group; in particular, the 20 mg/kg QOW group had fewer infusion reactions than the 40 mg/kg QOW group.

**Dose-Response:** The potential relationship between alglucosidase alfa dose (20 mg/kg versus 40 mg/kg QOW) and response (invasive ventilator-free survival and any ventilator-free survival) was assessed. Comparison of subjects receiving 20 mg/kg QOW doses versus those receiving 40 mg/kg QOW doses revealed no differences with regard to these response assessments. With regard to safety, subjects receiving the higher dose experienced more infusion-associated reactions (IARs).

**Pharmacodynamic Findings:** Pharmacodynamic measurements included skeletal muscle GAA activity, skeletal muscle glycogen content, and glucose tetrasaccharides (Hex₄) in plasma and urine. Skeletal muscle GAA activity increased with time from Baseline to Week 12 in subjects receiving alglucosidase alfa 20 or 40 mg/kg QOW, with increases greater in the subjects receiving 40 mg/kg QOW compared to those receiving 20 mg/kg QOW. There was no clear trend of skeletal muscle glycogen content with time (Baseline, Week 12, and Week 52) or with dose (20 mg/kg or 40 mg/kg QOW). Plasma Hex₄ and urine Hex₄ appeared to decrease with time (Baseline, Week 4, Week 12, and Week 26), but no clear difference between the two doses (20 mg/kg and 40 mg/kg) was appreciated.

**Immunogenicity:** The majority of patients (34 of 38; 89.5%) in the two clinical trials tested positive for IgG antibodies to alglucosidase alfa. One patient was seropositive at baseline, 15 patients seroconverted by Week 4, 8 patients seroconverted by Week 8, and 3 patients seroconverted by Week 12. Antibody titers appeared to plateau by about 8 weeks after starting therapy. Titers appeared to be higher in the 40 mg/kg dose group compared to the 20 mg/kg dose group. Nineteen of 21 patients who received treatment with alglucosidase alfa and had pharmacokinetics and antibody titer data available at Week 12 developed antibodies to alglucosidase alfa. Five patients with higher antibody titers (≥12,800) at Week 12 had an average increase in CL of 51% (range 4% to 90%) from Day 1 to Week 12. The other 14 patients with lower antibody titers (<12,800) at week 12 had similar average CL values at Day 1 and Week 12. Based on the 11 PK evaluable patients in the pivotal trial, antibody titer appeared to be inversely related with invasive ventilator-free survival. The four patients with higher antibody titers (≥12,800) had invasive ventilator-free survival of 25%. The seven patients with lower antibody titers (<12,800) had invasive ventilator-free survival of 71%. IARs (overall) were 46.2% (18/39). Sustained IARs were 53% (8/15) in those with positive antibody titers, and none (0/3) in those with negative antibody titers.

**Genetic Information:** The mutation class (maternal/maternal alleles) were too numerous to attempt association with outcome; these included inframe deletion/frameshift, missense/missense, nonsense/nonsense, splice site/frameshift, missense/frameshift, missense/splice site, frameshift/frameshift, and inframe deletion/inframe deletion. Cross-reacting Immunologic Material positive or CRIM(+) status (defined as patient having the 110 KD precursor or intermediate cleavage products of GAA) was associated with higher invasive ventilator-free survival and lower peak antibody titers than CRIM(-) patients. CRIM(+) patients had invasive ventilator-free survival of 73.3% (11/15) and peak antibody titers of median (range) 3,200 (0 to 204,800); CRIM(-) patients had invasive ventilator-free survival of none (0/3) and peak antibody titers of median (range) 204,800 (51,200-409,600).

**Single-Dose PK Parameters at Various Dose Levels:** The pharmacokinetics of alglucosidase alfa were studied in 13 infantile-onset Pompe disease patients aged 1 to 7
months after a single infusion of 20 mg/kg IV (n=5) over approximately 4 hours, and after a single infusion of 40 mg/kg IV (n=8) over approximately 6.5 hours. Plasma levels of GAA were determined based on an activity assay using an artificial substrate. Maximum serum concentration (Cmax) and area under the curve (AUC) increased in a nearly dose proportional manner, 1.7 times and 2.2 times, respectively, as the dose increased from 20 mg/kg to 40 mg/kg. Clearance (CL) appeared to be independent of dose; mean CL was 25.2 and 24.1 mL/hr/kg at doses of 20 mg/kg and 40 mg/kg respectively. As the dose increased from 20 to 40 mg/kg, the mean Vss increased from 96.3 to 119.0 mL/kg (increase of 25%) and the mean t1/2 increased from 2.3 to 2.9 hours (increase of 24%). Another single dose PK study was conducted in 14 infantile-onset Pompe disease patients aged 6 to 43 months after IV infusion of 20 mg/kg over approximately 4 hours; the PK parameter values were similar to those in the 1 to 7 months age group study.

**Multiple-Dose PK:** After administration of 20 mg/kg IV (n=5) or 40 mg/kg IV (n=8) every other week for 12 weeks to all patients who participated in the above-mentioned single dose PK study, the PK parameters after the seventh dose (week 12) and after the first dose were comparable. Mean CL was 22.1 and 24.2 mL/hr/kg after the seventh dose of 20 mg/kg and 40 mg/kg, respectively.

**Drug Metabolism and Drug-Drug Interaction:** No studies on the metabolism of alglucosidase alfa have been performed in humans or in animals. Alglucosidase alfa is a recombinant human protein and is expected to be metabolically degraded through peptide hydrolysis. Based on its expected metabolism, alglucosidase alfa is an unlikely candidate for cytochrome P450 mediated drug-drug interactions. No drug-drug interaction studies were performed between alglucosidase alfa and concomitant medications.

**Pharmacokinetics in Special Populations:** In clinical studies with pharmacokinetics data (n=28 patients), median (range) age at first infusion was 7.2 months (range: 1.2 to 43.1 months). Based on this limited number of subjects, no obvious differences in pharmacokinetics were appreciated by gender (19 male, 9 female), body weight (range of 3.4 to 14 kg), race (15 Caucasian, 5 Black, 4 Asian, 4 Hispanic), and age (range of 1.2 to 43.1 months at first infusion). No formal studies were conducted to examine the effects of renal or hepatic impairment on the pharmacokinetics of alglucosidase alfa.

**Inter-Individual Variability in PK Data:** The mean coefficient of variation of CL and Vss was 38% and 33%, respectively, as determined by noncompartmental analysis in the combined dataset of subjects of age ranging from 1.2 to 43.1 months.

**Comparability among Product Lots:** The Sponsor initially proposed to market both the 160-L scale product and the 2000-L scale product of alglucosidase alfa in the original BLA submission. Only the 160-L scale product was used in the clinical pivotal trial. Pharmacokinetic comparability between the 160-L scale and 2000-L scale products was studied in GAA knockout mice; the results failed to demonstrate comparability between the 2000-L scale product and the 160-L scale product. The 2000-L scale product had a 30% lower plasma AUC than the 160-L scale product. The 2000-L scale product had 28 to 81% higher liver uptake and 20 to 65% lower quadriceps uptake between 1 to 8 hours after dosing. Based on these results, the sponsor later withdrew the 2000-L scale product from this BLA submission. Preliminary PK data in five patients using the 2000-L scale product from an ongoing study was submitted in an amendment; conclusions regarding PK comparability of the 2000-L scale product and the 160-L scale product cannot be made from
the results of that data because of the limited number of patients (n=5), and the different patient population studied (age range 5 to 15 years) from that in the clinical pivotal trial (age range 1 to 7 months). The PK data from that study will be submitted along with other study results using the 2000-L scale product in a BLA supplement.

Adverse Events: Serious and important adverse reactions associated with alglucosidase alfa were most often infusion associated reactions (IARs). IARs were reported in 18 of 39 patients (46.2%) treated with alglucosidase alfa. IARs were more commonly reported in the 40 mg/kg group (n=9), with 123 IARs compared to 41 IARs in the 20 mg/kg group (n=9). The majority of AEs (95%) were reported to be mild or moderate in severity. The most frequent severe events included respiratory failure (16), pneumonia (8), pyrexia (3), respiratory arrest (2), respiratory distress (2), cardiorespiratory arrest (2), oxygen saturation decreased (2), sputum retention (2), bradycardia (2), hypotension (2), ejection fraction decreased (2), dysphagia (2), and atelectasis (2). Similar numbers of SAEs occurred in the 20 mg/kg dose and 40 mg/kg dose groups in the pivotal trial.
Reviewer, Team Leader, and Division Director Signatures

Anil Rajpal, M.D.
Clinical Pharmacology Reviewer

Hong Zhao, Ph.D.
Team Leader, Biologics Team

Shiew-Mei Huang, Ph.D.
Division Director (Acting), Office of Clinical Pharmacology Division 5

3/23/06
2. Question-Based Review (QBR)

2.1 General Attributes

1. What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product? What is the proposed mechanism of drug action and therapeutic indication? What is the proposed dosage and route of administration?

**Chemistry and Physical-Chemical Properties:** Alglucosidase alfa is produced by recombinant DNA technology in a Chinese hamster ovary cell line. Alglucosidase alfa is a glycoprotein with a calculated mass of 99,377 daltons (excluding the mass of the carbohydrates). The recombinant protein contains Alglucosidase alfa has a specific activity of 3-5 U/mg (one unit is defined as that amount of activity that results in the hydrolysis of 1 μmole of synthetic substrate per minute under the assay conditions).

**Formulation:** Alglucosidase alfa is a sterile, nonpyrogenic, white to off-white, lyophilized cake or powder for reconstitution with 10.3 mL Sterile Water for Injection, USP. Each 50 mg vial contains 5 mg alglucosidase alfa, 210 mg mannitol, 0.5 mg polysorbate 80, 9.9 mg sodium phosphate dibasic heptahydrate, 31.2 mg sodium phosphate monobasic monohydrate. Following reconstitution as directed, each vial contains 10.5 mL reconstituted solution and a total extractable volume of 10 mL at 5.0 mg/mL alglucosidase alfa.

**Mechanism of Action:** Pompe disease is an inherited, progressive muscle disease resulting from a deficiency of the lysosomal enzyme GAA. Deficiency of GAA results in the accumulation of organelle-bound (lysosomal) and extra lysosomal glycogen. Alglucosidase alfa degrades glycogen by catalyzing the hydrolysis of α-1,4- and α-1,6- glycosidic linkages of lysosomal glycogen. Glycogen accumulation in Pompe disease occurs in various tissues, particularly cardiac, respiratory and skeletal muscle, leading to the development of cardiomyopathy and progressive muscle weakness, including impairment of respiratory function. Alglucosidase alfa is intended to provide an exogenous source of GAA for patients with Pompe disease.

**Indication:** The sponsor's proposed indication for alglucosidase alfa is The pivotal clinical data submitted is from the infantile-onset Pompe disease population.

**Dosage and Route of Administration:** The recommended dose of alglucosidase alfa is 20 mg/kg body weight administered every 2 weeks as an approximately 4-hour intravenous infusion. In clinical trials, doses up to 40 mg/kg have been administered every 2 weeks. The total volume of infusion is determined by the patient's body weight and should be administered over approximately 4 hours for the 20 mg/kg dosage and over approximately 6.5 hours for the 40 mg/kg dose. In the clinical trials, pre-treatment medications were used but not routinely administered to patients. Infusions should be administered in a step-wise manner using an infusion pump. The initial infusion rate should be no more than 1 mg/kg/hr. The infusion rate
may be increased by 2 mg/kg/hr every 30 minutes, after patient tolerance to the infusion rate is established, until a maximum rate of 7 mg/kg/hr is reached. The infusion rate may be slowed and/or temporarily stopped in the event of infusion reactions.

2. What efficacy and safety information (e.g., biomarkers, surrogate endpoints, and clinical endpoints) contribute to the assessment of clinical pharmacology and biopharmaceutics study data (e.g., if disparate efficacy measurements or adverse event reports can be attributed to intrinsic or extrinsic factors that alter drug exposure/response relationships in patients)?

Invasive Ventilator-free Survival: As the primary efficacy endpoint, it was measured as the proportion of patients in the single arm pivotal trial who were alive and free of invasive ventilator support at 18 months of age as compared to a historical control.

Any Ventilator-free Survival: As a secondary efficacy endpoint, it was evaluated as the proportion of patients in the single arm pivotal trial who were alive and free of any ventilatory support (invasive or non-invasive) at 18 months of age, as compared to a historical control.

Left Ventricular Mass Index (LVMI): Changes in LVMI from Baseline to Week 26 were evaluated via echocardiography measurements.

Infusion Associated Reactions (IARs): IARs were defined as those AEs occurring on the day of infusion from the onset of the infusion up to and including the 2-hour observation period and were assessed by the Investigator as being at least possibly related to alglucosidase alfa.

2.2 General Clinical Pharmacology

1. What is the basis for selecting the response endpoints, i.e., clinical or surrogate endpoints, or biomarkers (also called pharmacodynamics, PD) and how are they measured in clinical pharmacology and clinical studies?

Invasive Ventilator-free Survival: Due to the progressive weakening of respiratory muscles, respiratory failure develops in patients with Pompe disease. Pulmonary function was evaluated by assessment of invasive ventilator-free survival (i.e., survival free of invasive ventilation). Invasive ventilation was defined as any ventilatory support applied with the use of an endotracheal tube or tracheostomy. For the clinical studies, patients were considered invasive ventilator-free at any time point (t) if they were free of invasive ventilation for the 14 day period bracketing the analysis time point.

Any Ventilator-free Survival: Pulmonary function was evaluated by assessment of any ventilator-free survival. (i.e. survival free of invasive or noninvasive ventilation). Noninvasive ventilation was defined as any form of ventilatory support applied without the use of an endotracheal tube. For the clinical studies, patients were considered any ventilator-free at any time point (t) if they were free of invasive and noninvasive ventilation for the 14 day period bracketing the analysis time point.

Left Ventricular Mass Index (LVMI): Infantile-onset Pompe disease is characterized by a progressive cardiomyopathy that results in heart failure. Left ventricular hypertrophy, expressed as abnormal left ventricular mass, is characteristic of the disease and longitudinal
measurements are commonly measured by echocardiography and used as an indicator of disease progression.

Skeletal Muscle Glycogen Content: The deficiency of lysosomal acid α-glucosidase (GAA) leads to accumulation of glycogen in skeletal muscle and other muscle tissues. Skeletal muscle glycogen content was measured at Baseline and at Week 12 in the pivotal study (Study 1602), and at Baseline, Week 12, and Week 52, in a supportive study (Study 1702).

Skeletal Muscle GAA Activity: It was measured at Baseline and at Week 12 in the pivotal study (Study 1602), and at Baseline, Week 12, and Week 52, in a supportive study (Study 1702).

Oligosaccharide levels: Oligosaccharide levels were measured in plasma and urine at Baseline and at Weeks 4, 12, and 26 in Study 1602 as a potential noninvasive method of monitoring disease status and response to α-glucosidase alpha therapy. In plasma, a tetraenzyme oligomer (designated Glc₄) as well as maltotetraose (designated M₄) are elevated, while in urine the vast majority (> 92%) of glucose tetraccharides are Glc₄. Previous studies indicated that Glc₄ and M₄ are formed through degradation of glycogen released into the circulation by the enzyme amylase. Thus, it is hypothesized that Hex₄ levels may correlate with the degree of glycogen accumulation in tissues of patients with Pompe disease.

2. **Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationship? (If yes, refer to IV, F, Analytical Section; if no, describe the reasons)**

Plasma and Skeletal Muscle rhGAA Content: The recombinant human acid alpha-glucosidase (rhGAA) content in human plasma as well as skeletal muscle biopsy samples were measured based on an activity assay using 4-methylumbelliferylalpha-D-glucopyranoside (4-MUG, as substrate. 4-MUG upon hydrolysis produces the fluorescent 4-methylumbelliflerone (4-MU).

Biochemical Analysis of Skeletal Muscle Glycogen Content: Glycogen content in skeletal muscle biopsies was measured using a 2-step biochemical procedure. After digesting the glycogen present in muscle tissues with amyloglucosidase, glucose was quantified.

Histomorphometric Analysis of Skeletal Muscle Glycogen Content: An image analysis system is used to measure glycogen content in a 2-dimensional histological section of muscle tissue.

Plasma Hex₄ and Urine Hex₄: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) with stable isotope dilution was used to analyze oligosaccharides Hex₄ in plasma and urine.

3. **What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy and safety?**

**Dose-Response:**

**Selected Clinical Response Measures:**
The safety and efficacy of 20 and 40 mg/kg alglucosidase alfa administered QOW was examined in the pivotal study, Study 1602. The 18 patients in this study were randomized in a 1:1 ratio to receive either 20 or 40 mg/kg QOW. Analyses did not reveal any differences between the two dose groups with respect to invasive ventilator-free survival, or any ventilator-free survival. (see Table 1)

**Table 1. Effect of 20 and 40 mg/kg QOW Alglucosidase Alfa on Selected Clinical Response Measures (Study 1602)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>20 mg/kg dose (n=9)</th>
<th>40 mg/kg dose (n=9)</th>
<th>Overall (n=18)</th>
<th>Historical Control (n=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients alive and free of invasive ventilation at 18 months of age¹</td>
<td>8 (89%)</td>
<td>7 (78%)</td>
<td>15 (83.3%)</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>Patients alive and free of any ventilation at 18 months of age²</td>
<td>6 (66.7%)</td>
<td>6 (66.7%)</td>
<td>12 (66.7%)</td>
<td>1 (1.6%)</td>
</tr>
</tbody>
</table>

¹ Patients younger than 18 months as of 15 September 2005 were censored in the analyses of invasive ventilator-free survival, and any ventilator-free survival.

² (Values in the table above are taken from the Review of the Clinical Reviewer Anne Parisor, M.D.)

The 18-month invasive ventilator-free survival rate of 83.3% (95% CI = 59, 96) for the alglucosidase alfa treated patients was approximately 52 times the 18-month survival rate for untreated historical controls of 1.6% (95% CI = 0.0, 5.5) with no overlap of the 95% CIs. The 18-month any ventilator-free survival rate for the alglucosidase alfa treated patients of 66.7% (95% CI = 45, 88) was approximately 42 times the 18-month survival rate for untreated historical controls of 1.6% (95% CI = 0.0, 5.5) with no overlap of the 95% CIs.

**Left Ventricular Mass Index (LVMI):**

Change in LVMI from Baseline to Week 26 was similar between the two dose groups. (see Table 2)

**Table 2. Change from Baseline to Week 26 in LVMI in Each Alglucosidase Alfa Dose Group (Study 1602)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose Group</th>
<th>Baseline</th>
<th>Week 26</th>
<th>Change from Baseline ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVMI (g/m²)</td>
<td>20 mg/kg (n=8*)</td>
<td>197.2 ± 58.5</td>
<td>95.2 ± 27.6</td>
<td>-50.3 ± 10.7</td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td>40 mg/kg (n=7)</td>
<td>189.1 ± 72.5</td>
<td>101.2 ± 42.4</td>
<td>-55.7 ± 19.8</td>
</tr>
</tbody>
</table>

* n=7 at Week 26

(Values in the table above were taken from the Sponsor's Clinical Study Report for Study 1602)

**Skeletal Muscle GAA activity:**

In Study 1602, change in skeletal muscle GAA activity from Baseline to Week 12 was significantly higher (p=0.014) in the 40 mg/kg dose group than the 20 mg/kg dose group (mean 342.1 nmol/hr/g tissue versus 98.7 nmol/hr/g tissue). (see Figure 1)
In Study 1702, skeletal muscle GAA activity change from Day 1 to Week 12 was similar to that for the 20 mg/kg dose group of Study 1602. At Week 52, skeletal muscle GAA activity appeared to increase further from Week 12 but less in magnitude.

**Skeletal Muscle Glycogen Content:**

Skeletal muscle glycogen content (biochemical) versus time and skeletal muscle glycogen content (histomorphometric) versus time are shown in the figure below for three groups of patients, those in Study 1602 receiving 20 mg/kg QOW, those in Study 1602 receiving 40 mg/kg QOW, and those in Study 1702 receiving 20 mg/kg QOW. (see Figure 2)
Figure 2. Skeletal Muscle Glycogen Content (Median) versus Time

In both Studies 1602 and 1702, there was no clear trend of skeletal muscle glycogen content with dose (20 mg/kg QOW or 40 mg/kg QOW) or with time since starting treatment (Baseline, 12 Weeks, or 52 Weeks).

Oligosaccharides – Plasma and Urine Hex₄

Plasma Hex₄ and Urine Hex₄ versus time is shown in the figure below. (see Figure 3)

Figure 3. Plasma Hex₄ (Median) and Urine Hex₄ (Median) versus Time

(Graphs above generated using Sponsor's data.)
Median Plasma Hex4 and Urine Hex4 appeared to decrease with time (from Baseline to Week 26) but no clear difference between the two dose levels (20 mg/kg QOW and 40 mg/kg QOW) was appreciated.

Effect of Immunogenicity on Clinical Outcome:

Invasive ventilator-free survival of the 11 PK evaluable patients in the pivotal trial (Study 1602) by antibody titer (≥ 12,800 vs. < 12,800) is shown in the table below. (see Table 3)

<table>
<thead>
<tr>
<th>Ab Titer (Wk 12)</th>
<th>Invasive Ventilator Free Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (≥ 12800) (n=4)</td>
<td>1 / 4 (25%)</td>
</tr>
<tr>
<td>Low (&lt; 12800) (n=7)</td>
<td>5 / 7 (71%)</td>
</tr>
</tbody>
</table>

IARs (overall) were 18/39 (46.2%). Sustained IARs of patients with positive antibody titer versus those with negative antibody titer are shown below. (see Table 4)

<table>
<thead>
<tr>
<th>Sustained IARs vs. Ab Titer (Studies 1602 and 1702)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) Ab titer</td>
</tr>
<tr>
<td>(-) Ab titer</td>
</tr>
</tbody>
</table>

a) Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

As the dose increased from 20 to 40 mg/kg in Study 1602, the Cmax and AUC increased in a nearly dose proportional manner, 1.7 times and 2.2 times, respectively. CL did not appear to change, and half-life (t1/2) appeared to increase by 25% as the dose increased from 20 to 40 mg/kg. (see Table 5)

<table>
<thead>
<tr>
<th>Ratio (40/20)</th>
<th>Cmax</th>
<th>AUC_{0-\infty}</th>
<th>t_{1/2}</th>
<th>CL</th>
<th>Vss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.71</td>
<td>2.20</td>
<td>1.25</td>
<td>0.96</td>
<td>1.24</td>
</tr>
</tbody>
</table>

b) Do PK parameters change with time following chronic dosing?

No. PK parameters did not appear to change with time following chronic dosing as shown below. (see Table 6)

<table>
<thead>
<tr>
<th>Study</th>
<th>Dose Group</th>
<th>Cmax</th>
<th>AUC_{0-\infty}</th>
<th>t_{1/2}</th>
<th>CL</th>
<th>Vss</th>
</tr>
</thead>
<tbody>
<tr>
<td>1602</td>
<td>20 mg/kg QOW (n=5)</td>
<td>1.31</td>
<td>1.29</td>
<td>1.04</td>
<td>0.88</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg QOW (n=8)</td>
<td>0.93</td>
<td>0.96</td>
<td>1.02</td>
<td>1.01</td>
<td>1.07</td>
</tr>
<tr>
<td>1702</td>
<td>20 mg/kg QOW (n=12)</td>
<td>0.99</td>
<td>1.15</td>
<td>1.27</td>
<td>0.87</td>
<td>0.97</td>
</tr>
</tbody>
</table>
c) **How long is the time to the onset and offset of the pharmacological response or clinical endpoint?**

The time to onset of the pharmacological response may be as early as 4-8 weeks. Based on echocardiographic measurements of LVMI, changes from Baseline > 2 SE in mean LVMI values were at Week 4 in Study 1602 and at Week 8 in Study 1702. The time to offset of the pharmacological response was not studied; patients are not taken off alglucosidase alfa because it is believed to prolong survival.

d) **Are the dose and dosing regimen consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?**

As the dose increased from 20 mg/kg QOW to 40 mg/kg QOW, the AUC approximately doubled. Analyses did not reveal any differences between the 2 dose groups, 20 mg/kg QOW and 40 mg/kg QOW, with respect to survival, invasive ventilator-free survival, any ventilator-free survival, or left ventricular mass index. A similarly high proportion of patients in the 20 and 40 mg/kg dose groups demonstrated clinical responses on each of these endpoints.

e) **How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?**

The pharmacokinetic information for intravenously administered alglucosidase alfa submitted in this application was obtained from 30 patients with Pompe Disease in two studies. No studies were conducted in healthy volunteers.

No studies on the metabolism of alglucosidase alfa have been performed in humans or in animals. Alglucosidase alfa is a recombinant human protein and is expected to be metabolically degraded through peptide hydrolysis to amino acids which are not considered as active metabolites.

f) **What are the basic PK parameters?**

**Single-dose PK Parameters**

<table>
<thead>
<tr>
<th>Study</th>
<th>Dose [mg/kg]</th>
<th>N</th>
<th>Cmax [mcg/mL]</th>
<th>AUC [mcg/hr/mL]</th>
<th>t1/2,α [hr]</th>
<th>Cl [mL/hr/kg]</th>
<th>Vss [mL/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1602</td>
<td>20</td>
<td>5</td>
<td>162 ± 31</td>
<td>811 ± 141</td>
<td>2.3 ± 0.4</td>
<td>25.2 ± 3.8</td>
<td>96.3 ± 15.7</td>
</tr>
<tr>
<td>1602</td>
<td>40</td>
<td>8</td>
<td>276 ± 64</td>
<td>1781 ± 520</td>
<td>2.9 ± 0.5</td>
<td>24.1 ± 6.5</td>
<td>119.0 ± 28.1</td>
</tr>
<tr>
<td>1702</td>
<td>20</td>
<td>14</td>
<td>188 ± 83</td>
<td>901 ± 314</td>
<td>2.1 ± 0.5</td>
<td>26.1 ± 14.2</td>
<td>84.8 ± 29.6</td>
</tr>
</tbody>
</table>

Values above were calculated by this reviewer from concentration time data in dataset PKX_PL_1 PK 1602 using WinNonlin. Note: Plasma GAA levels were determined based on an activity assay using an artificial substrate.

Following a 4-hour infusion of 20 mg/kg, Cmax and AUC increased in a nearly dose proportional manner, 1.7 times and 2.2 times, respectively, as the dose increased from 20 to 40 mg/kg. Cl appeared to be independent of dose. Following a 4-hour infusion of 20 mg/kg, single-dose PK parameters in Study 1702 were similar to those in Study 1602.
After infusion of 20 mg/kg QOW or 40 mg/kg QOW, pharmacokinetic parameters after the seventh infusion were similar to those after the first infusion. After infusion of 20 mg/kg QOW, multiple-dose PK parameters in Study 1702 were similar to those in Study 1602.

**g) Is this a high extraction ratio or a low extraction ratio drug?**

Not applicable.

**h) Does mass balance study suggest renal or hepatic the major route of elimination?**

No mass balance study has been conducted for alglucosidase alfa. Alglucosidase alfa is a recombinant enzyme. Mass balance studies are not generally performed for recombinant enzymes because these are proteins which are degraded into amino acids that are then recycled into other proteins.

### 2.3 Intrinsic Factors

1. **What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics?**

   **Pharmacokinetics in Special Populations:** The oldest patient in studies with PK data (n=28) was 43.1 months at the time of the first infusion. Other open-label clinical trials of alglucosidase alfa have been performed in older pediatric patients ranging from 2 to 16 years at the initiation of treatment, but data was not submitted with the original BLA submission. The safety and effectiveness of alglucosidase alfa in elderly patients has not been established.

2. **Based upon what is known about exposure-response relationships and their variability, and the groups studied (volunteers vs. patients); what dosage regimen adjustments, if any, are recommended for each of these subgroups (examples shown below)? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.**

   **a) Pediatric Patients**

   All of the 28 patients with PK data were pediatric patients. Median (range) age at the time of the first infusion was 7 months (range: 1.2 to 43.1 months). Correlation was found between CL (estimated in mL/hr/kg) and age after the first infusion with p value of 0.005; however, after removal of the outlier with CL value of 67.9 mL/hr/kg, no significant correlation between CL (estimated in mL/hr/kg) after the first infusion and age was found with p value of 0.59. No
significant correlation was found between CL (estimated in mL/hr/kg) and age after the Week 12 infusion with p value of 0.67. (see Figure 4)

Figure 4. CL versus Age (Day 1 and Week 12)

PK parameters in Studies 1602 and 1702 are compared below. In Study 1602, median (range) age at first infusion was 5.7 months (1.2 to 7.3 months). In Study 1702, median (range) age at first infusion was 14.0 months (6.3 to 43.1 months). No significant differences in PK parameters between the two studies were appreciated. (see Table 9)

Table 9. Ratio of Mean PK Parameters in Study 1702 to Study 1602

<table>
<thead>
<tr>
<th></th>
<th>Ratio of Mean PK Parameters in Study 1702 to Study 1602</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cmax</td>
</tr>
<tr>
<td>Single-dose (20 mg/kg)</td>
<td>1.16</td>
</tr>
<tr>
<td>Multiple-dose (20 mg/kg QOW X 6)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

b) Body Weight

Dose of alglucosidase alfa is based on body weight. Median (range) body weight in the 28 patients with PK data was 6.7 kg (range: 3.4-16.0 kg).

Correlation was found between CL (estimated in mL/hr/kg) and body weight after the first infusion with p value of 0.045; however, after removal of the outlier with CL value of 67.9 mL/hr/kg, no significant correlation between CL (estimated in mL/hr/kg) after the first infusion and body weight was found with p value of 0.65. No significant correlation was found between CL (estimated in mL/hr/kg) and age after the Week 12 infusion with p value of 0.33. (see Figure 5)
c) Gender

Of the total 28 patients with PK data, 19 patients were male, and 9 patients were female. No significant correlation between CL and gender was found after Day 1 infusion or after Week 12 infusion with p value of 0.16 and 0.82, respectively. After removal of the outlier with CL value of 67.9 mL/hr/kg, no significant correlation between CL and gender was found after Day 1 infusion with p value of 0.59. (see Figure 6)
d) Race

Of the total 28 patients with PK data, 15 patients were Caucasian, 5 patients were Black, 4 patients were Asian, and 4 were Hispanic. No significant correlation between CL and race was appreciated after Day 1 infusion or after Week 12 infusion with p value of 0.72 and 0.65, respectively. After removal of the outlier with CL value of 67.9 mL/hr/kg, no significant correlation between CL and race was found after Day 1 infusion with p value of 0.45. (see Figure 7)

Figure 7. CL versus Race (Day 1 and Week 12)

<table>
<thead>
<tr>
<th>CL versus Race (Day 1)</th>
<th>CL versus Race (Week 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASIAN</td>
<td>BLACK</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>RACE</td>
<td></td>
</tr>
</tbody>
</table>

e) Renal Impairment

No formal studies were conducted to examine the effects of renal impairment on the pharmacokinetics of alglucosidase alfa.

f) Hepatic Impairment

No formal studies were conducted to examine the effects of hepatic impairment on the pharmacokinetics of alglucosidase alfa.

g) What pregnancy and lactation use information is there in the application?

The following statements are proposed to be included in the label:

**Pregnancy Category B:** Reproduction studies have been performed in mice at doses of alglucosidase alfa up to 40 mg/kg administered daily for 10 days with no evidence of embryofetal abnormality. There are no adequate and well-controlled studies of alglucosidase alfa in pregnant women. Because animal reproduction studies are not always predictive of human response, alglucosidase alfa should be used during pregnancy only if clearly needed.

**Nursing Mothers:** It is not known whether alglucosidase alfa is secreted in human milk. Because many drugs are secreted in human milk, caution should be exercised when alglucosidase alfa is administered to a nursing woman.
h) **Other factors that are important to understanding the drug's efficacy and safety**

i. **Immunogenicity:**

Serum samples for IgG anti-rhGAA antibody testing were obtained pre-infusion, at 4-week intervals for the first 26 weeks, then at Weeks 36 and 52, and at 12-week intervals thereafter in both Studies 1602 and 1702. Across both studies, 21 patients that received treatment with alglucosidase alfa had pharmacokinetics and IgG antibody titer data available at Week 12. Of these 21 patients, 19 developed IgG antibodies to alglucosidase alfa by Week 12. There appeared to be a trend of patients with higher IgG antibody titers having increases in CL from Day 1 to Week 12. (see Figure 8)

![Figure 8. % Change in CL (Day 1 to Week 12) versus Antibody Titer at Week 12](image)

(Figure above generated by this reviewer using data from the Sponsor.)

Five patients with high antibody titers (≥ 12800) at week 12 had an average increase in CL of 51% (range 4% to 90%) from day 1 to week 12. The other 14 patients with low antibody titers (<12,800) at week 12 had similar average CL values at day 1 and week 12.
ii. Infusion Associated Reactions:

Infusion Associated Reactions (IARs) were more commonly reported in the 40 mg/kg group (n=9), with 123 IARs compared to 41 IARs in the 20 mg/kg group (n=9). (Numbers of IARs taken from Review of Clinical Reviewer Anne Pariser M.D.) Across both studies 1602 and 1702, IARs were reported in 18 of 39 patients (46.2%) treated with alglucosidase alfa.

The most common IARs included urticaria, flushing, pyrexia and rash. All infusion reactions were reported as mild or moderate in severity. Less common infusion reactions included rales, bronchospasm, and periorbital edema. Serious infusion reactions included urticaria, rales, tachycardia, decreased oxygen saturation, bronchospasm, tachypnea, periorbital edema, and hypertension all of which occurred in individual patients, except urticaria (2 patients). Some patients were pre-treated with antihistamines and/or antipyretics in order to manage infusion reactions. Infusion reactions may occur at any time during the infusion of alglucosidase alfa, especially with higher infusion rates.

If an infusion reaction occurs, regardless of pre-treatment, decreasing the infusion rate, temporarily stopping the infusion, and/or administration of antihistamines and/or antipyretics may ameliorate the symptoms. If severe hypersensitivity or anaphylactic reactions occur, immediate discontinuation of the administration of alglucosidase alfa should be considered and appropriate medical treatment should be initiated.

iii. Cardiac Toxicity and Related Disorders:

Precaution must be observed when administering general anesthesia to patients with infantile-onset Pompe disease. Reports of intraoperative cardiac arrest following anesthesia induction for invasive procedures have been reported, some of which were fatal. The presence of severe hypertrophic cardiomyopathy in infantile-onset Pompe disease may increase the risk of general anesthesia complications. While these specific events are clearly unrelated to the administration of alglucosidase alfa, it underscores the need for caution in the use of general anesthesia in this patient population.

iv. Genetic Information:

The Sponsor indicated the intent of using genetic and genomic studies was for research purposes only in order to assess the efficacy of alglucosidase infusion as enzyme replacement therapy. The gene for GAA is on chromosome 17q25. Over 70 mutations have been identified in the literature. Nonsense and frameshift mutations are associated with higher risk, and patients with these mutations typically have absent GAA. Missense mutations are associated with lower risk, and patients with these mutations typically have low levels of GAA. Other mutations include deletion, insertion, and splice site mutation.

In this submission, the mutation class (maternal/paternal Alleles) were too numerous to attempt association with outcome. These included inframe deletion/frameshift, missense/missense, nonsense/nonsense, splice site/frameshift, missense/frameshift, missense/splice site, frameshift/frameshift, and inframe deletion/infame deletion.

Cross-reacting Immunologic Material (CRIM) status is defined as follows: CRIM(+) if patient has the 110 KD precursor or intermediate cleavage products of GAA. CRIM(+) status is associated with a more favorable outcome. In the pivotal clinical study (Study 1602), the
results of invasive ventilator-free survival and peak antibody titers by CRIM status are shown below. (see Table 10)

**Table 10. Invasive Ventilator-Free Survival and Peak Antibody Titers (Study 1602)**

<table>
<thead>
<tr>
<th>CRIM Status</th>
<th>Invasive Ventilator-Free Survival (%)</th>
<th>Peak Antibody Titers Median (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRIM (+)</td>
<td>73.3% (11/15)</td>
<td>3,200 (0 to 204,800)</td>
</tr>
<tr>
<td>CRIM (-)</td>
<td>0% (0/3)</td>
<td>204,800 (51,200-409,600)</td>
</tr>
</tbody>
</table>

CRIM (+) patients had higher invasive ventilator-free survival and lower peak antibody titers than CRIM(-) patients.

v. **Other:**

Patients should be informed that a registry for patients with Pompe disease has been established in order to better understand the variability and progression of Pompe disease and to continue to monitor and evaluate treatments. Patients should be encouraged to participate and advised that their participation may involve long-term follow-up.

2.4 **Extrinsic Factors**

1. **What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?**

Extrinsic factors such as drugs, herbal products, diet, smoking, and alcohol use have not been studied.

2. **Based upon what is known about exposure-response relationships and their variability, what dosage regimen adjustments, if any, do you recommend for each of these factors? If dosage regimen adjustments across factors are not based on the exposure-response relationships, describe the basis for the recommendation.**

None.

3. **Drug-Drug interactions**

a) **Is there an in vitro basis to suspect in vivo drug-drug interaction? Is the drug a substrate of CYP enzymes? Is the drug an inhibitor and/or an inducer of CYP enzymes? Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes? Are there other metabolic/transporter pathways that may be important?**

No studies on the metabolism of alglucosidase alfa have been performed in humans or in animals. Metabolism studies are not generally performed for biotechnology-derived pharmaceuticals because these are proteins which are degraded into amino acids that are then recycled into other proteins. Several pathways have been described that may contribute to protein metabolism, all of which involve biodegradation of the protein to smaller molecules, i.e., small peptides or amino acids. This fact has been recognized in ICH Topic S6 (Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, dated
July 16, 1997), where it is stated, “The expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids.” and “Classic biotransformation studies as performed for pharmaceuticals are not needed.” No in vitro drug-drug interaction studies have been performed since the P450 enzyme system is not expected to play any role in alglucosidase alfa biotransformation.

b) Does the label specify co-administration of another drug (e.g., combination therapy in oncology) and if so, has the interaction potential between these drugs been evaluated? No.

c) What other co-medications are likely to be administered to the target patient population?

Concomitant medications with frequency greater than 33% in the clinical studies included acetaminophen, albuterol, amoxicillin / clavulenic acid, ketamine, heparin, ibuprofen, midazolam, amoxicillin, budesonide, fentanyl, atropine, chloral hydrate, digoxin, nystatin, pneumococcal conjugate vaccine, salbutamol sulfate, cefazolin, hydrocortisone, furosemide, lidocaine, methylprednisolone, and vancomycin. Effect of individual or combined concomitant medications on PK of alglucosidase alfa was not studied.

d) Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?

In vivo drug-drug interaction studies have not been performed.

e) Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any? No known mechanistic basis for pharmacodynamic drug-drug interactions is known at this time.

f) Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions or protein binding?
None at this time.

2.5 General Biopharmaceutics

1. What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

The proposed to-be marketed products in the original BLA submission were the 160-L scale product and the 2000-L scale product. The sponsor withdrew the 2000-L scale product from this BLA submission based on the results of PK comparability studies between the 160-L scale and 2000-L scale products that were conducted in GAA knockout mice (presented below).

A listing of 160-L scale lots used in Study 1602 and Study 1702 is provided below.
Study 1602: Lots 930018, 608345, 608341, 531710, 751295, and 996793
Study 1702: Lots 930018, 608345, 608341
2000-L Scale versus 160-L Scale

Study 05-0414: PK Comparability Study

Pharmacokinetic comparability between the 160-L scale clinical trial drug product (one lot) and the 2000-L scale to-be-marketed product (two lots) was investigated in a study in GAA knockout mice, Study 05-0414. In that study, a single 20 mg/kg IV dose was given, and 12 animals were assigned to each of the three lots. Results are shown below. (see Table 11 and Figure 9)

Table 11. Ratio (AUC\textsubscript{0-\textinfty} of 2000 L Scale Lots) / (AUC\textsubscript{0-\textinfty} of 160 L Scale Lot)

<table>
<thead>
<tr>
<th>Lot Number</th>
<th>Ratio (AUC\textsubscript{0-\textinfty} of 2000 L Scale Lots) / (AUC\textsubscript{0-\textinfty} of 160 L Scale Lot 930118)</th>
<th>Point Estimate</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5744693</td>
<td>0.76</td>
<td>63.3 % to 88.3 %</td>
<td></td>
</tr>
<tr>
<td>4573352</td>
<td>0.64</td>
<td>54.3 % to 76.6 %</td>
<td></td>
</tr>
</tbody>
</table>

Note: Plasma GAA levels were determined based on an activity assay using an artificial substrate.

Figure 9. Pharmacokinetics of 2000 L and 160 L rhGAA in GAA Knockout Mice (05-0414)

(Figure below is taken from the Sponsor's Study Report for 05-0414Pga)

Pharmacokinetics of 2000 L and 160 L rhGAA in GAA Knockout Mice

(Pharmacia 05-0414Pga)

The 90% CI of test (2000-L) to reference (160-L) for AUC\textsubscript{0-\textinfty} fell below the limits of 80 to 125%. For the first 2000-L scale lot, 90% CI was 63.3 to 88.3 % (Point estimate=0.76). For the second 2000 L scale lot, 90% CI was 54.3 to 75.6 % (Point estimate=0.64). Thus, the exposure as measured by AUC\textsubscript{0-\textinfty} is approximately 30% less for the two 2000-L scale lots compared to the 160-L scale lot.

Study 05-0252: Biodistribution Study

Biodistribution of the 2000 L scale product (two lots) was compared to biodistribution of the 160 L scale product in Study 05-0252. In that study, a single dose of 20 mg/kg IV was given, and 9 animals were assigned to each lot. Results are shown below. (see Figure 10, Table 12, and Table 13)
Figure 10. Biodistribution of 2000 L and 160 L rhGAA in GAA Knockout Mice (05-0252)

Table 12. Liver: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot

<table>
<thead>
<tr>
<th>Liver: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot</th>
<th>2000 L Lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
<th>160 L Lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5744693</td>
<td>128 %</td>
<td>157 %</td>
<td>141 %</td>
<td>930018</td>
<td>100 %</td>
<td>100 %</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>4573352</td>
<td>181 %</td>
<td>166 %</td>
<td>138 %</td>
<td>4573352</td>
<td>100 %</td>
<td>100 %</td>
<td>100 %</td>
<td></td>
</tr>
</tbody>
</table>

Table 13. Quadriceps: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot

<table>
<thead>
<tr>
<th>Quadriceps: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L Lot</th>
<th>2000 L lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
<th>160 L Lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>5744693</td>
<td>35 %</td>
<td>86 %</td>
<td>80 %</td>
<td>930018</td>
<td>100 %</td>
<td>100 %</td>
<td>100 %</td>
<td></td>
</tr>
<tr>
<td>4573352</td>
<td>35 %</td>
<td>57 %</td>
<td>80 %</td>
<td>4573352</td>
<td>100 %</td>
<td>100 %</td>
<td>100 %</td>
<td></td>
</tr>
</tbody>
</table>

In the liver, there appears to be higher GAA activity in those mice administered the 2000 L scale lots as compared to those administered the 160 L scale lot. In the quadriceps, there appears to be lower GAA activity in those mice administered the 2000 L scale lots as compared to those administered the 160 L scale lot. In the spleen and triceps, consistent differences for the 3 lots over the 3 time points were not appreciated.

The sponsor has withdrawn the 2000 L scale product from this BLA.

Preliminary PK data in five patients from an ongoing study (Study 2804) using the 2000-L scale product was submitted as an amendment. Conclusions regarding PK comparability of the 2000-L scale product and the 160-L scale product cannot be made from the results of that
study because of the limited number of patients (n=5), and the different population studied (age range 5 to 15 years) from that in the clinical pivotal trial (age range 1 to 7 months).

a) What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

The 2000-L scale product may have lower efficacy than the pivotal clinical trial scale product (160-L scale) because of the approximately 30% lower systemic exposure observed in a PK comparability study in GAA knockout mice.

b) If the formulation does not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to-be-marketed product?

Approval of the pivotal clinical trial scale product (160-L scale) only is recommended. A qualified potency assay with pharmacological relevance for alglucosidase alfa, and clinical safety and efficacy data are required for supporting the approval of the 2000-L scale product.

c) If the formulations are not BE, what dosing recommendations should be made that would allow approval of the to-be-marketed formulation? (e.g., dosage adjustments may be made for injectables)

The 2000-L scale product should not be approved. Only the clinical trial 160-L product should be approved. The sponsor has agreed to only market the 160-L scale product.

2. What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types? When would a fed BE study be appropriate and was one conducted? How do the dissolution conditions and specifications assure in vivo performance and quality of the product?

Not applicable because alglucosidase alfa is given via intravenous infusion.

2.6 Analytical Section

1. What are measured in the plasma in the clinical pharmacology and biopharmaceutics studies?

- Plasma rhGAA Content
- Skeletal muscle GAA activity
- Skeletal muscle glycogen content (by biochemical analysis)
- Histomorphometric analysis of skeletal muscle glycogen content
- Plasma Hex₄
- Urine Hex₄
2. Which metabolites have been selected for analysis and why?

None. No studies on the metabolism of alglucosidase alfa have been performed in humans or in animals. Alglucosidase alfa is a recombinant human protein and is expected to be metabolically degraded through peptide hydrolysis. Based on its expected metabolism, alglucosidase alfa is an unlikely candidate for cytochrome P450 mediated drug-drug interactions.

3. For all moieties measured, is free, bound or total measured? What is the basis for that decision, if any, and is it appropriate?

Not applicable because alglucosidase alfa is a protein.

4. What bioanalytical methods are used to assess concentrations?

**Plasma rhGAA Content:** The recombinant human acid alpha-glucosidase (rhGAA) content was measured in human plasma samples using 4-methylumbelliferylalpha-D-glucopyranoside (4-MUG), as substrate. 4-MUG is an artificial substrate. 4-MUG upon hydrolysis produces the fluorescent 4-methylumbelliferone (4-MU). The overall precision of interpolated enzyme mass values of the samples ranged from 9.7 to 15.2% coefficient of variation. The linear range of the method was 2.5 to 500 ng/mL, with a limit of quantification of 12.5 ng/mL.

**Skeletal Muscle GAA Activity:** Frozen muscle biopsies were homogenized, sonicated, and then centrifuged. An aliquot of the resulting supernatant was used to assay GAA activity by the 4-MUG assay, which was carried out in skeletal muscle tissue homogenates. As described for plasma samples, 4-MUG upon hydrolysis produces the fluorescent 4-methylumbelliferone (4-MU). The assay does not discriminate between lysosomal and nonlysosomal GAA that is active at an acidic pH. Assay quantitation limit was 0.333 nmol/h/mL. Range of linearity for clinical trial material (CTM), human foreskin fibroblasts (HFF), and peripheral blood mononuclear cell (PBMC) was 0.75 to 121.4 nmol/h/mL, 0.90 to 115.7 nmol/h/mL, and 0.31 to 26.24 nmol/h/mL, respectively.

**Biochemical Analysis of Skeletal Muscle Glycogen Content:** Glycogen content in skeletal muscle biopsies was measured using a 2-step biochemical procedure. After digesting the glycogen present in muscle tissues with amyloglucosidase, glucose was quantified. An assay performance study suggests that differences of up to 10% may be attributed to assay variability. Assay quantitation limit was 12.5 mg/dL glucose.

**Histomorphometric Analysis of Skeletal Muscle Glycogen Content:** An image analysis system is used to measure glycogen content in a 2-dimensional histological section of muscle tissue. Up to 10 slides are analyzed per patient time point. The mean glycogen content and SD for each set are then calculated. This number represents the average glycogen content for each patient sample. The average and SD for the patient time point can then be calculated and used in comparing pre- and post-treatment values to determine the change in glycogen content. A meaningful change in glycogen content was defined by Genzyme as an estimate (mean glycogen content ± 1 SD) that did not overlap with the Baseline estimate.
Plasma Hex₄ and Urine Hex₄: Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze oligosaccharides Hex₄ in plasma and urine.

According to the Product Reviewer, Dr. Frederick Mills, the sponsor has made progress toward developing the following qualified potency assays:

The Sponsor will use the new assays to assess potency and product quality for six of the 160 L alg glucosidase alfa lots used in clinical trials, as well as two 160 L Myozyme commercial launch lots. These analyses will be completed by March 24, 2006, and comprise a database that will be used to create specifications for manufacture of subsequent 160 L lots.

3. Detailed Labeling Recommendations

Sponsor’s Proposed Clinical Pharmacology: Pharmacokinetics, Effects of Antibodies Sections

Pharmacokinetics

In a pivotal trial including 18 patients, the pharmacokinetics of MYOZYME were evaluated in 15 patients with Pompe disease who received doses of 20 mg/kg or 40 mg/kg MYOZYME as an approximate 4 to 6.5-hour infusion, respectively. Pharmacokinetics were dose proportional and did not change over time. After the first and sixth infusion of MYOZYME, mean maximum plasma concentrations (Cmax) ranged from 178.2 to 263.7 µg/mL for the 20 mg/kg and 40 mg/kg dose groups respectively. The mean area under the plasma concentration-time curve (AUCₜ) ranged from 977.5 to 1,872.5 µg•hr/mL for the 20 mg/kg and 40 mg/kg dose groups. Mean plasma clearance (CL) was 21.9 mL/h/kg and mean volume of distribution at steady state (Vss) was 66.2 mL/kg for both dose groups with small between-subject variability of 15% and 11%, respectively. Mean plasma elimination half-life (t½) was 2.75 hours for the two dose groups.

The pharmacokinetics of MYOZYME were also evaluated in a separate trial in 21 patients with Pompe disease who received doses of 20 mg/kg of MYOZYME. In 12 patients with available data the AUCₜ and Cmax were approximately equivalent to those observed for the 20 mg/kg dose group in the pivotal trial. The t½ of approximately 2-3 hours was also similar in this group of patients.

Effects of Antibodies

Most patients who received infusions of MYOZYME developed antibodies to alg glucosidase alfa by week 12. The presence of antibodies did not appear to affect the pharmacokinetics of MYOZYME.

Reviewer’s Proposed Labeling in Clinical Pharmacology: Pharmacokinetics section

This reviewer proposes that Clinical Pharmacology: Effects of Antibodies not be a separate section, and be incorporated into Clinical Pharmacology: Pharmacokinetics as shown below because the effect of antibodies on pharmacokinetics is being described.
Pharmacokinetics

The pharmacokinetics of alglucosidase alfa were evaluated in 13 patients of age 7 months or less with infantile-onset Pompe disease who received 20 mg/kg (as an approximate 4-hour infusion) or 40 mg/kg (as an approximate 6.5-hour infusion) of TRADENAME every two weeks. The plasma concentration measurement of alglucosidase alfa was based on an activity assay using an artificial substrate. Systemic exposure was approximately dose proportional over the range of 20 to 40 mg/kg. (see Table 1)

Table 1. Pharmacokinetic Parameters (Mean ± SD) After Single 20 mg/kg or 40 mg/kg Intravenous Infusion of TRADENAME

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>20 mg/kg (n=5)</th>
<th>40 mg/kg (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mcg/mL)</td>
<td>162 ± 31</td>
<td>276 ± 64</td>
</tr>
<tr>
<td>AUC (mcg-hr/mL)</td>
<td>811 ± 141</td>
<td>1781 ± 520</td>
</tr>
<tr>
<td>CL (mL/hr/kg)</td>
<td>25 ± 4</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>Vss (mL/kg)</td>
<td>96 ± 16</td>
<td>119 ± 28</td>
</tr>
<tr>
<td>t½ (hr)</td>
<td>2.3 ± 0.4</td>
<td>2.9 ± 0.5</td>
</tr>
</tbody>
</table>

The pharmacokinetics of alglucosidase alfa were also evaluated in a separate trial in 14 patients of age ranging from 6 months to 3.5 years with infantile-onset Pompe disease who received 20 mg/kg of TRADENAME as an approximate 4-hour infusion every two weeks. The pharmacokinetic parameters were similar to those observed for the 20 mg/kg dose group in the trial of patients of age 7 months or less.

Nineteen of 21 patients who received treatment with TRADENAME and had pharmacokinetics and antibody titer data available at week 12 developed antibodies to alglucosidase alfa. Five patients with antibody titers ≥ 12800 at week 12 had an average increase in clearance of 50% (range 5% to 90%) from week 1 to week 12. The other 14 patients with antibody titers <12,800 at week 12 had similar average clearance values at week 1 and week 12.

Sponsor's Proposed Adverse Reactions: Immunogenicity Section (First Paragraph)

The majority of patients (34 of 38; 89.5%) in the two clinical trials tested positive for IgG antibodies to alglucosidase alfa.

Reviewer's Proposed Adverse Reactions: Immunogenicity Section (First Paragraph)

The majority of patients (34 of 38; 89.5%) in the two clinical trials tested positive for IgG antibodies to alglucosidase alfa.
Page(s) Withheld

§ 552(b)(4) Trade Secret / Confidential

§ 552(b)(5) Deliberative Process

§ 552(b)(4) Draft Labeling
4.2 Individual Study Reviews

4.2.1 Product Development Rationale

Pompe disease is a rare, autosomal recessive disease caused by the deficiency of lysosomal acid α-glucosidase (GAA), an enzyme that degrades glycogen. The resulting accumulation of glycogen in body tissues, especially cardiac, respiratory, and skeletal muscle, disrupts the architecture and function of affected cells leading to multisystemic pathology and death. In addition to being a lysosomal storage disorder (LSD), Pompe disease is also considered a neuromuscular disease, a metabolic myopathy, and a glycogen storage disorder. The estimated global incidence of Pompe disease is 1:40,000; however, variations in incidence have been reported between different ethnic groups.

Historically, Pompe disease has been classified into different subtypes based on age at onset of symptoms, extent of organ involvement, and rate of progression to death. Essentially, the disease manifests as a broad clinical spectrum with a continuum of symptoms, ranging from a rapidly progressive infantile-onset form to a more slowly progressive late-onset form with considerable variability and overlap between these 2 ends of the spectrum.

Age at symptom onset tends to correlate with residual GAA activity, which often correlates inversely with disease severity. Thus, in general, the later the onset of symptoms, the greater the residual GAA activity, the better the prognosis. However, exceptions to this rule exist, suggesting that residual GAA activity is not the sole determinant of clinical phenotype. Importantly, all presentations of Pompe disease share the same underlying deficiency of lysosomal GAA, leading to progressive tissue damage, and ultimately death.
At the most rapidly progressive end of the disease spectrum are patients with the infantile-onset form of Pompe disease. These patients typically present with signs and symptoms within the first 12 months of life. A massive accumulation of glycogen in the heart and skeletal muscle results in rapidly progressive cardiomyopathy and generalized muscle weakness with hypotonia. Motor development is often completely arrested, or if motor milestones are achieved, they are subsequently lost, and death from cardiac and/or respiratory failure occurs before most patients reach 1 year of age. Patients presenting with this typical disease course have been described in the literature as having "classic" infantile-onset Pompe disease. A subset of patients with infantile onset Pompe disease characterized by a slightly later age at onset (but before 12 months of age) and slower progression of cardiomyopathy has been described by Sionim and colleagues. These "nontypical" patients may survive beyond their first birthday, but typically develop respiratory failure between 1 to 2 years of age.

Currently, there is no approved treatment for Pompe disease and palliative and supportive care tend to be the mainstay of patient management, though they do not prevent disease progression. The Sponsor's proposed indication for alglucosidase alfa (recombinant human acid alpha-glucosidase, [rhGAA]; INN: alglucosidase alfa) is for

4.2.2 Clinical Pharmacology and Pharmacokinetics Program

**Overall Assessment of Clinical Pharmacology:** The PK profile of alglucosidase alfa has been characterized in two studies in patients with Pompe Disease. The pharmacokinetics of alglucosidase alfa is predictable. The incidence of antibodies to the product is high but does not appear to impact clinical outcome. In summary, based on all available safety, efficacy, PK/PD and immunogenicity data, the chosen dosing of an initial alglucosidase alfa dose of 20 mg/IV is safe and effective.

**Data:** The PK information contained in this application is based on serum alglucosidase alfa concentration data obtained from a total of 30 patients with Pompe disease. No studies in healthy subjects were performed with alglucosidase alfa.

<table>
<thead>
<tr>
<th>Data Source: Overview of Clinical Studies with PK Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose (IV QOW)</strong></td>
</tr>
<tr>
<td>20 mg/kg (n=6)</td>
</tr>
<tr>
<td>40 mg/kg (n=9)</td>
</tr>
<tr>
<td><strong>Median (Range) Age</strong></td>
</tr>
<tr>
<td><strong>Median (Range) Weight</strong></td>
</tr>
<tr>
<td><strong>Product Scale</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PK (Plasma GAA Activity)</strong></td>
</tr>
<tr>
<td><strong>Muscle GAA Activity</strong></td>
</tr>
<tr>
<td><strong>Muscle Glycogen Content</strong></td>
</tr>
<tr>
<td><strong>Oligosaccharide Levels (Urine,Plasma)</strong></td>
</tr>
<tr>
<td><strong>BL:</strong> Baseline</td>
</tr>
</tbody>
</table>

**Assay:** In Studies AGLU01602 and AGLU01702, the GAA activity of alglucosidase alfa in human plasma was measured by Genzyme using a 4-methylumbelliferyl-α-D-glucoside (4-MUG) substrate. This is a fluorometric assay used to detect GAA activity in plasma, quantified relative to the activity of an 8-point rhGAA standard curve prepared from a dilution series of reference rhGAA.
The concentration of rhGAA in the sample was determined by interpolation from the standard
curve and the data are expressed as units of concentration (ng/mL). Assay results were accepted
if assay controls containing rhGAA read within the established range.

4.2.3 Pharmacokinetics

Single and Multiple Dose PK

Single and multiple dose PK were studied in Studies AGLU1602 and AGLU1702. Study
AGLU1602 was a study of 20 mg/kg or 40 mg/kg IV given every other week for 12 weeks. Study
AGLU 1702 was a study of 20 mg/kg IV given every other week for 12 weeks.

Study AGLU01602

Title: A Randomized, Open-Label, Multicenter, Multinational, Dose-Ranging Study of the Safety,
Efficacy, Pharmacokinetics, and Pharmacodynamics of Recombinant Human Acid alpha-
Glucosidase (rhGAA) Treatment in Patients < 6 Months Old with Infantile-Onset Pompe
Disease (Glycogen Storage Disease Type II)

Methods:

This is a randomized, open-label, multicenter, multinational dose-ranging study of the safety,
efficacy, pharmacokinetics, and pharmacodynamics of alglucosidase alfa involving patients < 6
months of age (adjusted for gestation) at the time of their first infusion. Data presented are from
18 patients who were treated in this study for a period of 26 weeks.

The pharmacokinetics of rhGAA were evaluated by measuring GAA activity in plasma. The GAA
activity in human plasma was measured using a fluorometric assay with limit of quantitation (LOQ)
of 0.33 nmol/mL. PK samples were taken after Day 1 and after Week 12. Blood samples were
obtained immediately before the infusion, at the completion of the infusion (Time 0), and at 0.5, 1,
3, 6, and 12 hours post-infusion.

rhGAA concentration-time data were analyzed using compartmental methods under a nonlinear
mixed effects model paradigm wherein each pharmacokinetic parameter was an allometric power
function of weight. Once the best structural model was identified, the influence of dose, age, and
sex was examined. When the final pharmacokinetic model was determined, the empirical Bayes
estimates (EBEs) of each individual patient's pharmacokinetic parameters (e.g., clearance [CL],
central volume [V1], volume of distribution at steady-state [Vss], etc) were estimated. Secondary
parameters were calculated based on the primary pharmacokinetic parameters (area under the
plasma concentration-versus-time curve [AUC], half-life [T1/2], and Vss) or from direct observation
of the data (observed peak concentration [Cmax]). Repeated measures analysis of variance was
then used to determine if the rhGAA pharmacokinetics changed over time and whether rhGAA
antibodies affected rhGAA pharmacokinetics.

Results:

Of the 18 subjects treated (11 male and 7 female), 15 subjects (10 male and 5 female) are
included in the analysis. The median (range) age at first infusion for analyzed subjects was 5.7
months (range 1.2 to 7.3 months). For the purpose of the PK analysis, age was not adjusted for
gestation.
Mean rhGAA concentration-time profiles are shown in the figure below.

Values for various pharmacokinetic parameters at Day 1 and Week 12 are summarized in the table below.

| Summary of Pharmacokinetic Profile for GAA after IV Infusion of 20 mg/kg or 40 mg/kg QOW to Patients With Infantile-Onset Pompe Disease (n=15) |
|---|---|---|---|
| **PK Parameter** | **Day 1 (n=15)** | **Mean ± SD** | **Week 12 (n=15)** |
| Cmax (ng/mL) | | | |
| 20 mg/kg | 160,910 ± 27,598 | | 195,540 ± 73,190 |
| 40 mg/kg | 271,253 ± 61,251 | | 256,096 ± 50,920 |
| AUC (hr*ng/mL) | | | |
| 20 mg/kg | 937,896 ± 199,381 | | 1,017,118 ± 262,276 |
| 40 mg/kg | 1,883,581 ± 407,002 | | 1,861,479 ± 407,002 |
| tV/2 (hr) | 0.57 ± 0.081 | 0.59 ± 0.065 |
| t1/2 (hr) | 2.71 ± 0.58 | 2.80 ± 0.57 |
| CL (mL/hr) | 133 ± 41 | 154 ± 51 |
| (mL/h/kg) | 22.1 ± 4.2 | 21.8 ± 5.4 |
| V1 (mL) | 264 ± 8.7 | 308 ± 91 |
| (mL/kg) | 43.5 ± 8.4 | 43.5 ± 8.4 |
| Vss (mL) | 404 ± 116 | 469 ± 100 |
| (mL/kg) | 66.9 ± 10.3 | 67.0 ± 9.8 |

[Values in the table above were taken from Page 25 of the Sponsor's Clinical Pharmacology Summary] a. N=18 total in Study AGLU01602. N=15 PK evaluable patients. Patients 1602-312, 1602-314, and 1602-316 were excluded from the analysis because they did not have pharmacokinetic samples collected at Day 1 or Wk 12.
Data are reported as mean ± standard deviation (SD) from individual estimates, not model-predicted averages. Cmax was estimated based on direct examination of the observed data. AUC, \( T_{\text{max}} \), and \( T_{\frac{1}{2}} \) were secondary derived parameters based on model-predicted pharmacokinetic values. CL, V1, and Vss were estimated using the empirical Bayes estimate for each individual's pharmacokinetic parameters.

After cessation of the alglucosidase alfa infusion, rhGAA concentrations appeared to decline monophasically in some subjects and biphasically in others. The data were best described using a 2-compartment model with linear elimination from the central compartment. The only important covariate that influenced rhGAA pharmacokinetics was weight. The model adequately predicted observed plasma rhGAA concentrations. Pearson's correlation coefficient between observed and individual predicted values was 0.9687 (p < 0.0001). The pharmacokinetics of rhGAA were dose proportional and did not change over time.

None of the pharmacokinetic parameters examined was affected by visit, indicating that rhGAA pharmacokinetics did not change over time. AUC was affected by dose group (p < 0.0001) and weight (p < 0.0001), with the AUC 1.9 times higher in the 40 mg/kg dose group than the 20 mg/kg dose group (90% confidence interval [CI]: 1.6, 2.3). Cmax was affected only by dose group (p = 0.0018), with maximal concentrations in the 40 mg/kg dose group being 1.5 times higher than those in the 20 mg/kg dose group (90% CI: 1.3, 1.8). CL, V1, and Vss were affected only by weight (p < 0.02). Weight also affected \( t_{\frac{1}{2}} \) (p = 0.0099) but did not affect \( T_{\frac{1}{2}} \).

None of the pharmacokinetic parameters when expressed on a per-body-weight basis was affected by dose, weight, or visit. The least-squares geometric mean CL, V1, and Vss were 21.4 mL/h/kg (90% CI: 19.7, 23.3 mL/h) 42.8 mL/kg (90% CI: 39.4, 46.4 mL/kg), and 66.2 mL/kg (90% CI: 62.4, 70.3 mL/kg), respectively. Between-subject variability in CL, V1, and Vss corrected for body weight was 15%, 19%, and 11%, respectively.

**Study AGLU01702**

**Title:** An Open-Label, Multicenter, Multinational, Study of the Safety, Efficacy, Pharmacokinetics, and Pharmacodynamics of Recombinant Human Acid alpha-Glucosidase (rhGAA) Treatment in Patients ≥6 and ≤36 Months Old with Infantile-Onset Pompe Disease (Glycogen Storage Disease Type II)

**Methods:**

This is an open-label, multicenter, multinational study of the safety, efficacy, pharmacokinetics, and pharmacodynamics of alglucosidase alfa involving patients ≥6 and ≤36 months of age (adjusted for gestation) at the time of their first infusion. Data presented are from 15 patients who were treated in this study for a period of 52 weeks.

The pharmacokinetics of rhGAA were evaluated by measuring GAA activity in plasma. The GAA activity in human plasma was measured using a fluorometric assay with limit of quantitation (LOQ) of 0.33 nmol/mL. PK samples were taken after Day 1 and after Week 12.

Noncompartmental analysis was used. The following pharmacokinetic parameters were calculated: Cmax, time to Cmax (Tmax), \( t_{\frac{1}{2}} \), AUC from time 0 to the time of the last quantifiable concentration (AUC\(_{0-t}\)), AUC from time 0 to infinity (AUC\(_{0-\infty}\)), CL, volume of distribution during the
terminal phase after IV administration (Vz), Vss, and mean residence time (MRT). Due to the short 
t½ observed in Study AGLU01702 relative to the dosing frequency of once every 2 weeks, each 
infusion was treated as a single dose for purposes of pharmacokinetic analysis.

Results:

Of the 15 subjects treated (9 male and 6 female), 14 subjects (9 male and 5 female) had PK 
measurements at Day 1 and 12 subjects (8 male and 4 female) had PK measurements at Week 
12. The median (range) age at first infusion for analyzed subjects was 14.0 months (range 6.3 to 
43.1 months).

Mean rhGAA concentration-time profiles are shown in the figure below.

![Mean Plasma Concentration-Time Profile for rhGAA after Infusion of Alglucosidase Alfa 20 mg/kg IV (Study AGLU01702) (The figure below is taken from Page 29 of the Sponsor's Clinical Pharmacology Summary)]

The data in the figure are expressed as time since the start of the -4-hr infusion. Thus, the 0 time point reflects plasma GAA levels prior to the start of the infusion. The other points on this graph correspond to blood samples drawn at 0, 0.5, 1, 3, 6, and 12 hr after the end of the infusion. The data are expressed as the average of all patients.

Values for various pharmacokinetic parameters at Day 1 and Week 12 are summarized in the 
table below.

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Day 1 (n=14)</th>
<th>Week 12 (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>188,112 ± 83,402</td>
<td>208,239 ± 58,612</td>
</tr>
<tr>
<td>Tmax (hr)</td>
<td>3.82</td>
<td>3.83</td>
</tr>
<tr>
<td>AUC₇₅ (hr*ng/mL)</td>
<td>850,849 ± 373,329</td>
<td>1,070,015 ± 253,071</td>
</tr>
<tr>
<td>AUC∞ (hr*ng/mL)</td>
<td>901,074 ± 312,911</td>
<td>1,103,327 ± 277,549</td>
</tr>
<tr>
<td>t₁/₂ (hr)</td>
<td>2.05 ± 0.49</td>
<td>2.72 ± 0.64</td>
</tr>
<tr>
<td>CL (mL/hr/kg)</td>
<td>26.1 ± 14.2</td>
<td>19.5 ± 6.24</td>
</tr>
<tr>
<td>(mL/hr)</td>
<td>286 ± 248</td>
<td>204 ± 83.3</td>
</tr>
<tr>
<td>Vz (mL/kg)</td>
<td>70.6 ± 20.0</td>
<td>74.1 ± 20.1</td>
</tr>
<tr>
<td>(mL)</td>
<td>744 ± 412</td>
<td>768 ± 251</td>
</tr>
<tr>
<td>Vss (mL/kg)</td>
<td>84.8 ± 29.6</td>
<td>75.8 ± 20.8</td>
</tr>
<tr>
<td>(mL)</td>
<td>914 ± 592</td>
<td>795 ± 304</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>3.42 ± 0.51</td>
<td>3.94 ± 0.49</td>
</tr>
</tbody>
</table>

a. N=14 PK evaluable patients at Day 1. Patient 1702-414 had no available plasma GAA concentration data and was excluded from both the Day 1 and Week 12 analyses.
b. N=12 PK evaluable patients at Week 12. Pharmacokinetic data were not evaluated for patients 1702-408, 1702-409 and 1702-41 at Wk 12.
Mean plasma GAA concentrations were slightly higher at Week 12 than at Day 1, as shown in the figure above and the table above, but the difference was not statistically significant. Similar slight increases were observed in Cmax, AUC0-t, and AUCQ, as shown in the table above. The mean values for CL, Vz, and Vss were comparable on Day 1 and Week 12. A statistical comparison is shown in the table below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Geometric Mean Ratio1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
</tr>
<tr>
<td>Cmax(ng/mL)</td>
<td>100.01</td>
</tr>
<tr>
<td>AUC∞ (hr*ng/mL)</td>
<td>109.13</td>
</tr>
<tr>
<td>AUC0-t (hr*ng/mL)</td>
<td>107.48</td>
</tr>
<tr>
<td>T½β (hr)</td>
<td>119.42</td>
</tr>
<tr>
<td>CL (mL/hr/kg)</td>
<td>91.63</td>
</tr>
<tr>
<td>CL (mL/hr)</td>
<td>102.34</td>
</tr>
<tr>
<td>Vz (mL/kg)</td>
<td>109.43</td>
</tr>
<tr>
<td>Vz (mL)</td>
<td>122.21</td>
</tr>
<tr>
<td>Vss(mL/kg)</td>
<td>100.27</td>
</tr>
<tr>
<td>Vss(mL)</td>
<td>111.98</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>109.42</td>
</tr>
</tbody>
</table>

1 Ratio of Week 12 to Day 1. Based on analysis of natural log-transformed data.

As shown in the figure above, the CL and Vz values for individual patients overlapped almost completely, and the within-patient levels were highly consistent between study days.

The data suggest that the pharmacokinetic profile of alglucosidase alfa does not change after repeated exposures between Day 1 and Week 12.
Study AGLU02804 (26-Week Interim Study Report)

Title: A Single Centre, Open-Label, Bridging Study of the Safety, Pharmacokinetics and Efficacy of Recombinant Human Acid Alpha-Glucosidase (rhGAA) Treatment in Patients with Late-Onset Pompe Disease (Glycogen Storage Disease Type II)

Methods:

Study AGLU02804 is an ongoing, single center, open-label, European study of the safety, pharmacokinetics, and efficacy of Myozyme (recombinant human acid alpha-glucosidase (rhGAA)) in patients with late-onset Pompe disease. To be included in the study, patients had to be >5 and <18 years of age and have a confirmed diagnosis of Pompe disease including documentation of either acid α-glucosidase (GAA) gene mutations or deficient endogenous GAA activity.

Intravenous (IV) infusion of 20 mg/kg of Myozyme (produced in Chinese hamster ovary cells in 2000 L scale bioreactors) once every 2 weeks (qow) for 26 weeks.

Five patients (3 males, 2 females) were enrolled and treated with 20 mg/kg qow Myozyme under Protocol AGLU02804 for 26 weeks. All 5 patients had a confirmed diagnosis of late-onset Pompe disease (GAA deficiency and/or genotyping) between the ages of 13 months and 11.6 years. The median age at first Myozyme infusion was 12.7 years (range 5.9 to 15.2 years).

The pharmacokinetics of rhGAA were characterized after the first (Day 1), seventh (Week 12), and fourteenth (Week 26) infusion. Blood samples for measurement of rhGAA were collected before the start of the infusion, at the end of the infusion, and 0.5, 1, 3, 6, and 12 hours after the end of the infusion. The activity of rhGAA in human plasma was measured using a 4MU-α-D-glucopyranoside substrate and was quantified relative to the activity of an rhGAA standard curve prepared from a dilution series of reference rhGAA.

Results:

The pharmacokinetics results are shown below.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th>Week 12</th>
<th>Week 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng/mL)</td>
<td>306,921 ± 104,801</td>
<td>368,904 ± 63,629</td>
<td>310,883 ± 65,866</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>3.37</td>
<td>3.42</td>
<td>3.43</td>
</tr>
<tr>
<td>AUC₀₋₅ (h-ng/mL)</td>
<td>1,394,373 ± 187,724</td>
<td>1,639,116 ± 252,970</td>
<td>1,434,006 ± 230,417</td>
</tr>
<tr>
<td>AUC₅₋₋ (h-ng/mL)</td>
<td>1,435,034 ± 182,983</td>
<td>1,689,479 ± 252,296</td>
<td>1,471,771 ± 230,970</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>2.71 ± 0.36</td>
<td>2.88 ± 0.61</td>
<td>2.59 ± 0.23</td>
</tr>
<tr>
<td>CL (mL/h/kg)</td>
<td>14.1 ± 1.66</td>
<td>12.1 ± 1.89</td>
<td>13.9 ± 2.30</td>
</tr>
<tr>
<td>Vz (mL/kg)</td>
<td>55.2 ± 11.2</td>
<td>50.4 ± 14.4</td>
<td>52.1 ± 11.4</td>
</tr>
<tr>
<td>Vss (mL/kg)</td>
<td>56.0 ± 12.2</td>
<td>47.2 ± 9.66</td>
<td>53.8 ± 10.7</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>3.94 ± 0.50</td>
<td>3.90 ± 0.36</td>
<td>3.86 ± 0.22</td>
</tr>
</tbody>
</table>

1. Mean ± standard deviation except for Tmax for which the median is reported.

Analysis of PK data from the first 26 weeks of treatment with alglucosidase alfa manufactured at the 2000 L scale administered to patients with Pompe disease at a dose of 20 mg/kg qow did not reveal any clear differences in pharmacokinetic parameters between Day 1 and Weeks 12 and 26.
No conclusions regarding the PK comparability of the 160 L scale product and the 2000 L scale product can be made based on the results of this study because the number of patients is small (n=5) and because the population studied is different from that studied using the 160 L scale product in the pivotal clinical trial. The population studied in this trial is of age range 5 to 15 years, whereas in the clinical pivotal trial using the 160 L scale product the age range was 1 month to 7 months.

**Pharmacokinetics in Special Populations**

No formal clinical studies in patients with hepatic impairment, renal impairment or in elderly populations were conducted.

**Drug Metabolism and In vitro Drug-Drug Interaction Studies**

No studies on the metabolism of alglucosidase alfa have been performed in humans or in animals. Metabolism studies are not generally performed for monoclonal antibodies because they are proteins which are degraded into amino acids that are then recycled into other proteins. Several pathways have been described that may contribute to antibody metabolism, all of which involve biodegradation of the antibody to smaller molecules, i.e., small peptides or amino acids. This fact has been recognized in ICH Topic S6 (Note for Guidance on Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals, dated July 16, 1997), where it is stated, “the expected consequence of metabolism of biotechnology-derived pharmaceuticals is the degradation to small peptides and individual amino acids” and that classical biotransformation studies as performed for pharmaceuticals may not be needed. No in vitro drug-drug interaction studies have been performed since the P<sub>450</sub> enzyme system is not expected to play a role in alglucosidase alfa biotransformation.

**Drug-Drug Interaction Studies**

No drug-drug interaction studies were conducted.

**4.2.4 Pharmacodynamics**

In Study 1602, muscle GAA activity, muscle glycogen content, and oligosaccharide levels (urine, plasma) were studied. In Study 1702, muscle GAA activity, and muscle glycogen content were studied.

**Muscle GAA Activity**

**Methods (Studies AGLU 1602 and 1702):**

Frozen quadriceps muscle biopsies were homogenized, sonicated, and then centrifuged. An aliquot of the resulting supernatant was used to assay GAA activity by the 4-MUG assay, which was carried out in skeletal muscle tissue homogenates using a method similar to that described for plasma samples.

**Results (Studies AGLU 1602 and 1702):**
In Study 1602, the Skeletal Muscle GAA activity appears to increase more in the 40 mg/kg dose group than for the 20 mg/kg dose group. However, the numbers are small (n=9 in the 40 mg/kg group). In study 1702, the trend appears to be that the Skeletal Muscle GAA activity levels are increasing with time.

**Muscle Glycogen Content**

Two methods of analysis, biochemical and histomorphometric, were used.

**Glycogen Content (Biochemical) in Quadriceps Muscle**

The biochemical assay quantitatively measures glycogen concentration (expressed as mg glycogen/g wet tissue) in a 3-dimensional piece of tissue. Therefore, this method measures glycogen present in muscle as well as in non-muscle cell types (i.e., endothelium, smooth muscle, and peripheral nerve cells, among others) included in a biopsy sample. Glycogen content in skeletal muscle biopsies was measured by Genzyme using a 2-step biochemical procedure. After digesting the glycogen present in muscle tissues with amyloglucosidase, glucose was quantified with a Glucose Trinder Kit. Briefly, tissue samples were homogenized and then clarified by centrifugation and boiled. Control samples included buffer in place of the enzyme. The glucose produced was then measured by incubating the samples with Glucose Trinder Reagent. Absorbance was measured at 505 nm and quantified against a 7-point standard curve. Assay results were accepted if assay controls containing either glycogen or glucose read within the established range.
An assay performance study suggests that differences of up to 10% may be attributed to assay variability. Therefore, a meaningful change in glycogen content was defined by Genzyme as a difference of > 20% (i.e., twice the variability in the assay) from Baseline. Glycogen content was considered to be stable if subsequent measurements were within 20% of the Baseline measurement.

**Glycogen Content (Histomorphometric) in Quadriceps Muscle**

Histomorphometric analysis of glycogen content was performed using a proprietary image analysis system (MetaMorph®), which quantitatively measures glycogen in the area of a 2-dimensional histologic section of muscle tissue. Results are expressed as percent tissue area of muscle occupied by glycogen. The pathologist evaluating the tissue samples was blinded with respect to the identity of the patients and the timing of the biopsy sample. One representative field from each slide was photographed with a digital camera and acquired with photo image capture software. Each digital image was formatted at a fixed pixel density using Adobe® PhotoShop® software. Each digital image was then opened using the MetaMorph Imaging Processing and Analysis software for histomorphometric analysis. MetaMorph, performed on periodic acid-Schiff (PAS)-stained tissue samples, selects and quantifies all purple pixels corresponding to accumulated glycogen. This value is the numerator. MetaMorph then selects and quantifies all color (both purple as well as the remaining light blue muscle tissue). This value is the denominator. A percentage is then calculated from these 2 values. This result represents the percent area of all tissue represented in a given image, which is occupied by the abnormal glycogen accumulation. One representative field from each slide is evaluated. Up to 10 slides are analyzed per patient time point. The mean glycogen content and SD for each set are then calculated. This number represents the average glycogen content for each patient sample. The average and SD for the patient time point can then be calculated and used in comparing pre- and post-treatment values to determine the change in glycogen content.

**Results:**

<table>
<thead>
<tr>
<th>Comparison of Skeletal Muscle Glycogen Content – Biochemical (mg glycogen/g tissue) In Studies 1602 and 1702 [Median (Range)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1602 (n=18)</td>
</tr>
<tr>
<td>20 mg/kg (n=9)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Week 12</td>
</tr>
<tr>
<td>Week 52</td>
</tr>
</tbody>
</table>

In study 1602, no clear trend of skeletal muscle glycogen with dose was appreciated. In study 1702, the skeletal muscle glycogen appears to be decreasing with time.

<table>
<thead>
<tr>
<th>Comparison of Skeletal Muscle Glycogen Content – Histomorphometric (% tissue area occupied by glycogen) In Studies 1602 and 1702 [Median (Range)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1602 (n=18)</td>
</tr>
<tr>
<td>20 mg/kg (n=9)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Week 12</td>
</tr>
<tr>
<td>Week 52</td>
</tr>
</tbody>
</table>

In Study 1602, no clear trend of skeletal muscle glycogen with dose was appreciated. In Study 1702, the skeletal muscle glycogen appears to be decreasing with time.
Oligosaccharide Levels (Urine, Plasma)

Methods (Study AGLU 1602):

In Study AGLU01602, liquid chromatography-tandem mass spectrometry (LC-MS/MS) with stable isotope dilution was used to analyze oligosaccharides in plasma and urine. These measurements were performed at a central laboratory at Duke University Medical Center according to published and modified procedures.

Results:

| Oligosaccharides – Plasma and Urine Hex4 in Study 1602 [Median (Range)] |
|-------------------------------|---------------------|---------------------|---------------------|---------------------|
|                               | Plasma Hex4         | Urine Hex4          |                     |                     |
|                               | 20 mg/kg (n=9)      | 32.9 (8.7)          | 40 mg/kg (n=9)      | 37.9 (1.3)          |
| BL                             | 2.5                 | 3.1                 |                     |                     |
| Wk 4                           | 1.1                 | 1.0                 | 14.4                | 16.2                |
| Wk 12                          | 0.9                 | 1.3                 | 18.5                | 9.7                 |
| Wk 26                          | 0.7                 | 0.7                 | 13.5                | 11.6                |

Median Plasma Hex4 and Urine Hex4 appeared to decrease with time. No clear difference between the two doses (20 mg/kg and 40 mg/kg) was appreciated.

4.2.5 Animal Studies

The issue of comparability of the 160 L and the 2000 L product was investigated in studies in GAA knockout mice. Three relevant studies were identified. These were a pharmacokinetics study (05-0414), a pharmacodynamics study (05-0271), and a biodistribution study (05-0252).

Pharmacokinetics

Study 05-0414

Methods:

The objective of the study was to determine the PK of the 2000 L product (scale up) versus the 160 L product. The dose was 20 mg/kg (single IV dose). 12 animals got the 160 L lot, 12 animals got one 2000 L lot, and 12 animals got another 2000 L lot. The study design is summarized below.

<table>
<thead>
<tr>
<th>Treatment Administration</th>
<th>rhGAA scale / Lot No.</th>
<th>Dose Level (mg/kg)</th>
<th>Number of Animals (Sex)</th>
<th>Dosing Regimen</th>
<th>Bleed Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>160 L / lot 930018</td>
<td>20</td>
<td>12 (M/F)</td>
<td>Once (Day 1)</td>
<td>5, 15, 30, 60, 120, 240 and 480 minutes post-injection</td>
</tr>
<tr>
<td></td>
<td>2000 L / lot 5744693</td>
<td></td>
<td>12 (M/F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2000 L / lot 4573352</td>
<td></td>
<td>12 (M/F)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pharmacokinetics of 2000 L and 160 L rhGAA in GAA Knockout Mice (Genzyme 05-0414Pga)

Pharmacokinetic Parameters of rhGAA in GAA Knockout Mice (Genzyme 05-0414Pga)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HL_lambda_2 (min)</td>
<td>136.8 ± 39.3</td>
<td>115.5 ± 32.4</td>
<td>107.9 ± 17.5*</td>
</tr>
<tr>
<td>CL (mL/min/kg)</td>
<td>0.57 ± 0.17</td>
<td>0.73 ± 0.09*</td>
<td>0.87 ± 0.2*</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>163.8 ± 57.0</td>
<td>114.0 ± 26.1*</td>
<td>100.3 ± 13.2*</td>
</tr>
<tr>
<td>AUC_{0-infty} (min*ug/mL)</td>
<td>38530.5 ± 13144.2</td>
<td>27633.4 ± 3169.2*</td>
<td>24165.3 ± 5955.8*</td>
</tr>
<tr>
<td>AUC/dose (min*ug/mL/mg/kg)</td>
<td>1926.5 ± 657.2</td>
<td>1381.7 ± 158.5*</td>
<td>1208.3 ± 297.8*</td>
</tr>
<tr>
<td>Vss (mL/kg)</td>
<td>91.1 ± 39.8</td>
<td>82.6 ± 15.1</td>
<td>86.2 ± 17.8</td>
</tr>
<tr>
<td>Rsq</td>
<td>0.92 ± 0.14</td>
<td>0.98 ± 0.02</td>
<td>0.98 ± 0.01</td>
</tr>
</tbody>
</table>

90% CI of ratio to 160 L Parameter: AUC_{0-infty}

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>63.31</td>
<td>54.26</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>88.26</td>
<td>75.64</td>
<td></td>
</tr>
</tbody>
</table>

90% CI of ratio to 160 L Parameter: AUC last

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>64.95</td>
<td>57.06</td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>89.96</td>
<td>79.03</td>
<td></td>
</tr>
</tbody>
</table>

Students t-test: *p<0.05 between 2000 L lots and 160 L control

The 90% CI of test (2000 L) to reference (160 L) for AUC_{0-infty} falls below the 80 to 125% limits for both 2000 L test lots. For the first 2000 L lot, 90% CI is 63.3 to 88.3% (Point estimate=0.76). For the second 2000 L lot, 90% CI is 54.3 to 75.6% (Point estimate=0.64). Among the two lots, the average point estimate is 0.70. Thus, the exposure as measured by AUC_{0-infty} is approximately 30% less for the two 2000 L lots compared to the 160 L lot.
Pharmacodynamics

Study 05-0271

Methods:

The dose was 100 mg/kg IV Q week X 4 doses or vehicle. Two lots of the 2000 L scale product (Lot 4573352 and Lot 5744693) and one lot of the 160 L product (Lot 930018) were administered. The objective was to determine “efficacy” as measured by biochemical glycogen content.

The study design is summarized below. The sample for glycogen content was taken 7 days after the last dose.

<table>
<thead>
<tr>
<th>Genzyme Study #</th>
<th>Treatment Administration</th>
<th>Route</th>
<th>Dose Level (mg/kg)</th>
<th>Dose Regimen¹</th>
<th>Number of Animals per Group</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>05-0271Pga</td>
<td>Myozyme (160 L lot 930018 and 2000 L lots 4573352 and 5744693)</td>
<td>IV</td>
<td>0 (vehicle) or 100</td>
<td>Weekly for 4 doses</td>
<td>6</td>
<td>M/F</td>
</tr>
</tbody>
</table>

Results:

<table>
<thead>
<tr>
<th>05-0271 Glycogen Content - Biochemical (mg/g tissue); Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group³</td>
</tr>
<tr>
<td>vehicle</td>
</tr>
<tr>
<td>2000 L (Lot # 4573352)</td>
</tr>
<tr>
<td>2000 L (Lot # 5744693)</td>
</tr>
<tr>
<td>160 L (Lot # 930018)</td>
</tr>
</tbody>
</table>

³ In the 3 treatment groups, the dose was 100 mg/kg IV QW X 4.

No significant differences were noted in the Glycogen Content – Biochemical for 2000 L scale as compared to the 160 L scale.

Biodistribution

Study 05-0252

Methods:

The objective of this study was to determine the biodistribution of alglucosidase alfa manufactured by Genzyme using the 2000 L cell culture and purification process as compared to a lot manufactured at the 160 L scale. Two lots of 2000 L alglucosidase alfa (4573352 and 5744693) were chosen for comparison to a 160 L alglucosidase alfa control (lot 930018). Nine mice got each lot. The dose is 20 mg/kg IV X 1. The study design is summarized below.
### Treatment Administration

<table>
<thead>
<tr>
<th>Route</th>
<th>rhGAA scale / Lot No.</th>
<th>Dose Level (ug/kg)</th>
<th>Number of Animals (Sex)</th>
<th>Dosing Regimen</th>
<th>Tissue Collection Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>2000 L / lot 4573352</td>
<td>20</td>
<td>9 (M/F)</td>
<td>Once (Day 1)</td>
<td>1, 4 and 8 hours post injection</td>
</tr>
<tr>
<td></td>
<td>2000 L / lot 5744693</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>160 L / lot 930018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Results:

**Study 05-0252 4MU Activity Results, Liver**

- **Timepoints:** 1 Hour, 4 Hour, 8 Hour
- **Tissue:** Liver
- **rhGAA Activity (ug/kg):**
  - 1 Hour: 50,000, 250,000, 300,000
  - 4 Hour: 200,000
  - 8 Hour: 60,000

**Study 05-0252 4MU Activity Results, Spleen**

- **Timepoints:** 1 Hour, 4 Hour, 8 Hour
- **Tissue:** Spleen
- **rhGAA Activity (ug/kg):**
  - 1 Hour: 100,000
  - 4 Hour: 40,000
  - 8 Hour: 20,000

**Study 05-0252 4MU Activity Results, Quadriceps**

- **Timepoints:** 1 Hour, 4 Hour, 8 Hour
- **Tissue:** Quadriceps
- **rhGAA Activity (ug/kg):**
  - 1 Hour: 10,000, 20,000, 30,000
  - 4 Hour: 6,000, 8,000
  - 8 Hour: 4,000, 6,000

**Study 05-0252 4MU Activity Results, Triceps**

- **Timepoints:** 1 Hour, 4 Hour, 8 Hour
- **Tissue:** Triceps
- **rhGAA Activity (ug/kg):**
  - 1 Hour: 3,000, 6,000
  - 4 Hour: 1,500, 3,000
  - 8 Hour: 750, 1,500

### Liver: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L

<table>
<thead>
<tr>
<th>Lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5744693</td>
<td>128 %</td>
<td>157 %</td>
<td>141 %</td>
</tr>
<tr>
<td>4573352</td>
<td>181 %</td>
<td>165 %</td>
<td>138 %</td>
</tr>
</tbody>
</table>

### Quadriceps: Mean % Injected Dose 2000 L Lot / Mean % Injected Dose 160 L

<table>
<thead>
<tr>
<th>Lot</th>
<th>1 hr</th>
<th>4 hrs</th>
<th>8 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5744693</td>
<td>35 %</td>
<td>86 %</td>
<td>80 %</td>
</tr>
<tr>
<td>4573352</td>
<td>35 %</td>
<td>57 %</td>
<td>60 %</td>
</tr>
</tbody>
</table>
In the liver, there appears to be higher GAA activity in those mice administered the 2000 L scale lots as compared to those mice administered the 160 L scale lot. In the quadriceps, there appears to be lower GAA activity in those mice administered the 2000 L scale lots as compared to those administered the 160 L scale lot. In the spleen and triceps, consistent differences for the 3 lots over the 3 time points were not appreciated.

### 4.2.6 Exposure-Response

The potential relationship between alglucosidase alfa exposure and response was assessed by the effect of the 20 mg/kg versus 40 mg/kg QOW dose on overall survival, invasive ventilator-free survival, and any ventilator-free survival. Other response assessments included left ventricular mass index (assessed by echocardiography), measurements of, weight at Week 26, motor gains at Week 26 (assessed by Alberta Infant Motor Scale [AIMS] age-equivalent scores), motor gains at Week 26 (assessed by Developmental Motor Milestones), and gains in functional skills at Week 26 (assessed by Pompe Pediatric Evaluation of Disability Inventory [Pompe PEDI] Mobility Scaled Scores). Comparison of subjects receiving 20 mg/kg QOW doses and 40 mg/kg QOW doses revealed no differences with regard to these response assessments. With regard to safety, an equal number of patients in the two dose groups experienced infusion-associated reactions (IARs); however, subjects receiving the higher dose experienced more IARs.

Other pharmacodynamic measurements included skeletal muscle GAA activity (measured at Baseline, Week 12, and Week 52), skeletal muscle glycogen content (measured at Baseline, Week 12, and Week 52), and glucose tetrasaccharides in plasma and urine (plasma Hex1 and urine Hex4 measured at Baseline, Week 4, Week 12, and Week 26). Skeletal muscle GAA activity increased with time from Baseline to Week 12 in subjects receiving alglucosidase alfa 20 or 40 mg/kg QOW, with increases greater in the subjects receiving 40 mg/kg QOW compared to those receiving 20 mg/kg QOW. There was no clear trend of skeletal muscle glycogen content with time (Baseline, Week 12, and Week 52) or with dose (20 mg/kg or 40 mg/kg QOW). Plasma Hex4 and urine Hex4 appeared to decrease with time (Baseline, Week 4, Week 12, and Week 26), but no clear difference between the two doses (20 mg/kg and 40 mg/kg) was appreciated.

### 4.2.7 Dose-Finding Rationale

Patients previously treated with an earlier version of rhGAA, Synpac rhGAA, in other studies usually received a dose of 10 mg/kg weekly. While the clinical responses of patients receiving 10 mg/kg Synpac rhGAA per week were encouraging, it was believed that not all patients exhibited an optimal response to treatment and that higher doses may be warranted.

Alglucosidase alfa was found to deplete tissue glycogen in GAA knockout mice in a dose-dependent fashion in 3 nonclinical studies in GAA knockout mice, which included weekly dosing at 1, 5, 20, 60, and 100 mg/kg. Higher doses of alglucosidase alfa removed a greater proportion of stored glycogen in a wide variety of tissues, particularly from the heart.

QOW dosing with alglucosidase alfa appears to be at least as effective as weekly dosing based on two nonclinical depletion/re-accumulation studies conducted in GAA knockout mice using a dose of 100 mg/kg. One study evaluated glycogen depletion and re-accumulation following administration of a single dose of alglucosidase alfa, while the other study evaluated the effect of
4 weekly doses. Both studies showed that depletion of glycogen from the heart is better than from skeletal muscle after alglucosidase alfa administration, although the pattern of depletion/re-accumulation followed a similar time-course for both muscle types. After 4 weekly doses of alglucosidase alfa, glycogen levels were consistently maintained at their lowest level from approximately Day 3 through Day 21. Tissue glycogen levels subsequently increased gradually to Day 42 but remained well below the levels measured at study onset. Heart and diaphragm levels remained undetectable at Day 42. This time-course of re-accumulation suggests that dosing at 2-week intervals will coincide with the lowest point of glycogen levels in heart and skeletal muscles, and thus provide the greatest depletion of the substrate.

Clinical experience to date suggests that patients have tolerated 20 mg/kg rhGAA per week in 2 divided doses (10 mg/kg twice weekly) for extended periods of time. QOW dosing would be more convenient for patients and their families with the likely benefit of better compliance.

4.2.8 Immunogenicity

The majority of patients (34 of 38; 89.5%) in the two clinical trials tested positive for IgG antibodies to alglucosidase alfa. The clinical significance of antibodies to alglucosidase alfa is not known. No patients tested positive for inhibitory antibodies during the clinical trials.

Study 1602:

Assays:

Patient antibody response was initially evaluated by an ELISA to detect anti-rhGAA antibodies. Patient serum samples that were reactive in the ELISA at a dilution of 1:100 were subsequently evaluated by the radioimmunoprecipitation (RIP) assay to confirm the presence of IgG-specific antibodies. Patient antibody responses were further quantified by determining antibody titers by ELISA. A research assay was used to test for the presence of inhibitory antibodies to rhGAA in the serum samples of IgG seropositive patients. The presence of in vitro inhibitory antibodies is demonstrated by the interference in enzyme activity by the patient’s serum.

Results:

Anti-rhGAA IgG antibody titers for patients in Study AGLU01602 are plotted over time in the figure below.
Fifteen of the 18 patients developed IgG antibodies to algglucosidase alfa as assessed by ELISA and confirmed by RIP. Antibody titers appeared to be higher in the 40 mg/kg dose group compared to the 20 mg/kg dose group.

Seroconversion occurred in nine patients by Week 4, in another four patients by Week 8, and in one patient by Week 12. The seven patients (Patients 301, 303, 311, 313, 317, 318, and 319) with the highest anti-rGAA antibody titer levels (51,200 to 204,800) reported during the interim analysis period developed titers greater than 25,600 by Week 24. In 4 patients (Patients 308, 309, 310, and 316), anti-rGAA antibody titer levels did not rise above 400. Three patients (Patients 306, 312, and 315) remained seronegative at all time points tested. In 13 of the 15 patients who developed anti-rGAA antibodies, titer levels reached a plateau or declined during the interim analysis period. The two patients with the highest titer levels (Patients 303 and 313) continued to exhibit increased antibody titers throughout the interim analysis period.

Eleven of the 15 patient with positive antibody titers had PK evaluable data at Day 1 and Week 12. The distribution of CL from Day 1 to Week 12 is shown in the figure below, and the change in CL from Day 1 to Week 12 is listed with the antibody titer at week 12 in the table below.
Those subjects with increases in CL from Day 1 to Week 12 appeared to have higher antibody titers at Week 12 than those subjects with decreases in CL from Day 1 to Week 12.

A retrospective analysis for the presence of inhibitory antibodies was performed on serum samples collected from patients at Baseline, Week 4, Week 8, and Week 12, and Week 24. Assay performance studies and analysis of normal human donor samples suggest that values <10% inhibition may be attributed to assay variability. None of the 18 patients in Study AGLU01602 exhibited inhibitory antibody activity at levels ≥ 10% at any of the assessment time points. Therefore, no inhibitory antibody activity was detected in any of the 18 patients.

**Study 1702:**

**Assays:**

Patient antibody response was initially evaluated by an ELISA to detect anti-rhGAA antibodies. Patient serum samples that were reactive in the ELISA at a dilution of 1:100 were subsequently evaluated by the radioimmunoprecipitation (RIP) assay to confirm the presence of IgG-specific antibodies. Patient antibody responses were further quantified by determining antibody titers by ELISA. A research assay was used to test for the presence of inhibitory antibodies to rhGAA in
the serum samples of IgG seropositive patients. The presence of in vitro inhibitory antibodies is demonstrated by the interference in enzyme activity by the patient's serum.

Results:

Anti-rhGAA IgG antibody titers for patients in Study AGLU01702 are plotted over time in the figure below.

![Anti-rhGAA IgG Antibody Titers in Study AGLU01702](image)

Fourteen of the 15 patients developed IgG antibodies to alglucosidase alfa, as assessed by ELISA and confirmed by RIP.

One patient (Patient 407) was seropositive (i.e., RIP positive) at Baseline, which suggests pre-existing cross-reactivity. Seroconversion occurred in six of the remaining 13 patients by Week 4, in another four patients by Week 8, in another two patients by Week 12, and in one patient by Week 38. Three of the patients (Patients 405, 407, 413) developed anti-rhGAA IgG antibody titers >10,000 by Week 8; no other patients reached these levels during the 52-week treatment period. Of the ten other patients whose antibody titers remained less than 10,000 at all measurements during the 26-week treatment period, one showed a decrease in titer (Patient 406) at subsequent measurements with continued alglucosidase alfa administration. The sole seronegative patient (Patient 409) had only Baseline and Week 4 samples collected and analyzed for IgG antibodies to rhGAA. This patient died prior to the Week 8 time point; therefore, no additional samples were collected.

Eleven of the 14 patients with positive antibody titers had PK evaluable data at Day 1 and Week 12. The individual CL values at Day 1 and at Week 12 are shown in the figure below; the change in CL from Day 1 to Week 12 is listed with the antibody titer at week 12 in the table below.
Those subjects with increases in CL from Day 1 to Week 12 appeared to have higher antibody titers at Week 12 than those subjects with decreases in CL from Day to Week 12.

None of the first 15 treated patients exhibited inhibitory antibody activity at levels ≥ 10% at any of the assessments. Therefore, no inhibitory antibody activity was detected in the any of the first 15 treated patients over the first 52 weeks of the study.

**Conclusion:**
- Overall, the incidence of anti-rhGAA response in patients receiving alglucosidase alfa was high (89.5%).
- Although the data are limited, there does not appear to be any relationship between the appearance of antibodies to alglucosidase alfa and the efficacy of the molecule.
- Across both studies 1602 and 1702, those patients with increases in CL from Day 1 to Week 12 appeared to have higher antibody titers at Week 12 than those subjects with no changes in CL from Day to Week 12.
4.2.9 General Biopharmaceutics

The active drug substance in alglucosidase alfa is rhGAA, also known as alglucosidase alfa. The rhGAA in alglucosidase alfa is identical to a commonly occurring form of the human GAA. Alglucosidase alfa is provided as a sterile lyophilized powder for injection intended for reconstitution with Water for Injection (WFI). The proposed alglucosidase alfa commercial formulation is composed of 5 mg/mL rhGAA, mannitol, Polysorbate 80 in sodium phosphate. Alglucosidase alfa is administered as an intravenous infusion. It is intended for use as...

Alglucosidase alfa was initially manufactured by Genzyme at a 30 L/60 L production scale, but was subsequently scaled up to the 160 L and 2000 L bioreactor scales. The Sponsor intended to commercialize both the 160 L and 2000 L process scales. The commercial formulation was initially used for alglucosidase alfa produced at the 30 L/60 L scale. This 30 L/60 L material was administered to patients in Studies AGLU01702. The manufacturing process for alglucosidase alfa was subsequently scaled up to the 160 L production scale and was used in Study AGLU01602. All clinical lots produced at the 160 L scale used the proposed commercial formulation. Finally, the manufacturing process was again scaled up to the 2000 L production scale.

Single dose pharmacokinetic and biodistribution studies were conducted throughout the nonclinical development of alglucosidase alfa. Study 05-0414Pga evaluated alglucosidase alfa produced at the 2000 L scale. This study determined that pharmacokinetic comparability was not established between the 160L lot control and either of the 2000 L lots evaluated under the conditions of this study. In Genzyme Study 05-0252 there were significantly increased enzyme levels in the livers of mice administered 2000 L alglucosidase alfa as compared to the 160 L control. The 2000-L scale product had 28 to 81% higher liver uptake and 20 to 65% lower quadriceps uptake between 1 to 8 hours after dosing. The percent of injected dose was similar in the other organs evaluated.

The sponsor withdrew the 2000 L scale product from this BLA submission based on the above results. Preliminary clinical PK data in five patients from Study AGLU02804 (26-Week Interim Study Report) using the 2000 L scale process was submitted in an amendment to the BLA.
Summary of Myozyme Process Scales Used in the Clinical Development Program
(taken from page 8 of Sponsor's Summary of Bioanalytical Studies and Associated Analytical Methods)

Summary of Myozyme Process Scales Used in the Clinical Development Program for BLA*

<table>
<thead>
<tr>
<th>International Study Number</th>
<th>Study Design</th>
<th>30 L/60 L Lots</th>
<th>160 L Lots</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGLU01502</td>
<td>Phase 2/3 Randomized, Open-label, Dose-Ranging, Multicenter Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of rhGAA in infantile-onset patients</td>
<td>None</td>
<td>608341, 751295, 930018, 996793, 608345, 531710</td>
</tr>
<tr>
<td>AGLU01702</td>
<td>Phase 1/2 Open-label Multicenter Safety, Efficacy, Pharmacokinetics and Pharmacodynamic Study of rhGAA in infantile-onset patients</td>
<td>GA062, GA063, GA079</td>
<td>930018, 608341, 608145</td>
</tr>
<tr>
<td>AGLU02202</td>
<td>Expanded Access protocol in infantile-onset patients</td>
<td>GA123, GA124, GA137, GA139, GA140, GA155, GA160</td>
<td>531709, 608341, 751295, 996793</td>
</tr>
<tr>
<td>AGLU02303</td>
<td>Phase 2, Open-label Extension Study</td>
<td>GA063, GA079, GA080, GA095, GA096, GA108, GA124, GA140</td>
<td>930018 and 608341</td>
</tr>
<tr>
<td>AGLU1205-02 A4:5</td>
<td>Phase 2, Open-label Extension Study</td>
<td>GA095, GA108</td>
<td>608341, 608345</td>
</tr>
<tr>
<td>AGLU02403</td>
<td>Phase 2 Open-label Extension Study in a Single Patient</td>
<td>GA063, GA079, GA080, GA095, GA108 and GA140</td>
<td>None</td>
</tr>
<tr>
<td>AGLU02503</td>
<td>European Formal Late-Onset Expanded Access Program</td>
<td>None</td>
<td>608341, 608345</td>
</tr>
</tbody>
</table>

* Clinical data on 2000 L scale material is not yet available as this material was not used in the clinic until after the data cut off points for the CSRs.

30/60 L Drug Substance Lots and Content:
(taken from Attachment sent by Dr. Frederick Mills, Product Reviewer)

<table>
<thead>
<tr>
<th>Drug Substance Lot</th>
<th>Content [m/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GW10124098</td>
<td></td>
</tr>
<tr>
<td>GA025</td>
<td></td>
</tr>
<tr>
<td>GA042</td>
<td></td>
</tr>
<tr>
<td>GA061</td>
<td></td>
</tr>
<tr>
<td>GA075</td>
<td></td>
</tr>
<tr>
<td>GA094</td>
<td></td>
</tr>
<tr>
<td>GA108</td>
<td></td>
</tr>
<tr>
<td>GA122</td>
<td></td>
</tr>
<tr>
<td>GA138</td>
<td></td>
</tr>
<tr>
<td>GA154</td>
<td></td>
</tr>
</tbody>
</table>

125141/0
### Table of 30/60 L Scale Lots:
(taken from page 11 of Sponsor's Quality Overall Summary – Drug Product)

<table>
<thead>
<tr>
<th>Drug Substance Lot(s)</th>
<th>Formulated Drug Substance Lot</th>
<th>Formulated Drug Substance Lot</th>
<th>Drug Product Lot</th>
<th>Bioreactor Scale</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA015</td>
<td>GW10124098</td>
<td>N/A</td>
<td>N/A</td>
<td>60 L</td>
<td>Nonclinical Studies</td>
</tr>
<tr>
<td>GW10124096</td>
<td>GW10124096</td>
<td>N/A</td>
<td>GW10124106</td>
<td>60 L</td>
<td>Nonclinical Studies</td>
</tr>
<tr>
<td>GA026</td>
<td>GA028</td>
<td>N/A</td>
<td>GA028</td>
<td>30 L/60 L</td>
<td>Reference Standard (GAA1) - testing (60 L) Nonclinical Studies</td>
</tr>
<tr>
<td>GA042</td>
<td>GA042</td>
<td>N/A</td>
<td>GA043</td>
<td>30 L/60 L</td>
<td>Clinical Material Nonclinical Studies</td>
</tr>
<tr>
<td>GA061</td>
<td>GA061</td>
<td>N/A</td>
<td>GA062 GA063</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material Nonclinical Studies (Lot GA063)</td>
</tr>
<tr>
<td>GA078</td>
<td>GA078</td>
<td>N/A</td>
<td>GA079 GA080</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material</td>
</tr>
<tr>
<td>GA094</td>
<td>GA094</td>
<td>N/A</td>
<td>GA095 GA096</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material Nonclinical Studies</td>
</tr>
<tr>
<td>GA108</td>
<td>GA108</td>
<td>N/A</td>
<td>GA108</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material</td>
</tr>
<tr>
<td>GA122</td>
<td>GA122</td>
<td>N/A</td>
<td>GA123 GA124</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material</td>
</tr>
<tr>
<td>GA138</td>
<td>GA138</td>
<td>N/A</td>
<td>GA139 GA140</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material</td>
</tr>
<tr>
<td>GA154</td>
<td>GA154</td>
<td>N/A</td>
<td>GA155 GA156</td>
<td>30 L/60 L</td>
<td>Clinical Trial Material</td>
</tr>
</tbody>
</table>

1. The concentration of these materials was 10.2 mg/mL to accommodate nonclinical study requirements.
2. Not Applicable. A Formulated Drug Substance, Formulated Drug Substance Lot was not produced with this material.
3. An aliquot of GA015 was diluted to a target concentration of mannitol and lyophilized. At each stage, samples were tested for formal stability studies.
4. Formulated to contain final excipient concentrations (w/w) of mannitol and sodium phosphate, and lyophilized. All other lots formulated to contain final excipient concentrations (w/w) of mannitol and sodium phosphate.
5. Due to lyophilized in separate operations.
6. This material was reconstituted with WFI containing lyophilbate 80 mg/mL for clinical use. This 3000L lots occurred at Allston Landing. Information on the lyophilization of 2000 L material at 36 NVA is provided in Section 3.2.5.2.6.5.3.
### 160 L Drug Substance Lots
(taken from Attachment sent by Dr. Frederick Mills, Product Reviewer)

<table>
<thead>
<tr>
<th>Drug Substance Lot</th>
<th>Content (m/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02TP07</td>
<td></td>
</tr>
<tr>
<td>03TP006</td>
<td></td>
</tr>
<tr>
<td>03TP007</td>
<td></td>
</tr>
<tr>
<td>03TP008</td>
<td></td>
</tr>
<tr>
<td>03TP021</td>
<td></td>
</tr>
<tr>
<td>03TP022</td>
<td></td>
</tr>
<tr>
<td>03TP029</td>
<td></td>
</tr>
<tr>
<td>03TP033</td>
<td></td>
</tr>
<tr>
<td>03TP040</td>
<td></td>
</tr>
<tr>
<td>03TP041</td>
<td></td>
</tr>
<tr>
<td>03TP042</td>
<td></td>
</tr>
<tr>
<td>03TP048</td>
<td></td>
</tr>
<tr>
<td>03TP058</td>
<td></td>
</tr>
<tr>
<td>03TP059</td>
<td></td>
</tr>
<tr>
<td>04TP012</td>
<td></td>
</tr>
<tr>
<td>04TP021</td>
<td></td>
</tr>
<tr>
<td>04TP022</td>
<td></td>
</tr>
<tr>
<td>04TP031</td>
<td></td>
</tr>
<tr>
<td>04TP034</td>
<td></td>
</tr>
<tr>
<td>04TP037</td>
<td></td>
</tr>
<tr>
<td>04TP047</td>
<td></td>
</tr>
<tr>
<td>04TP049</td>
<td></td>
</tr>
<tr>
<td>04TP055</td>
<td></td>
</tr>
</tbody>
</table>

### Table of 160 L Scale Lots:
(taken from Sponsor's Quality Overall Summary – Drug Product)

<table>
<thead>
<tr>
<th>Drug Substance Lot(s)</th>
<th>Formulated Drug Substance Lot</th>
<th>Formulated Drug Substance Lot</th>
<th>Drug Product Lot</th>
<th>Bioreactor Scale</th>
<th>Use Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>02TP027</td>
<td>02TP028</td>
<td>N/A</td>
<td>930018</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>03TP006</td>
<td>03TP008</td>
<td>03TP014</td>
<td>803241</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>03TP009</td>
<td>03TP007</td>
<td>03TP021</td>
<td>902345</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>03TP012</td>
<td>03TP022</td>
<td>03TP023</td>
<td>751205</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>03TP015</td>
<td>03TP018</td>
<td>03TP025</td>
<td>920303</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>03TP018</td>
<td>03TP019</td>
<td>03TP030</td>
<td>531509</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>04TP006</td>
<td>04TP007</td>
<td>04TP012</td>
<td>531790</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>04TP011</td>
<td>04TP013</td>
<td>04TP014</td>
<td>1560817</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
<tr>
<td>04TP015</td>
<td>04TP016</td>
<td>04TP017</td>
<td>1560018</td>
<td>160 L</td>
<td>Clinical Trial Material Stability Studies Nonclinical Studies Process Validation</td>
</tr>
</tbody>
</table>

Not Applicable: A Formulated Drug Substance Lot was not produced with this material.
2000 L Drug Substance Lots,

(taken from Attachment sent by Dr. Frederick Mills, Product Reviewer)

<table>
<thead>
<tr>
<th>Drug Substance Lot</th>
<th>Content [m/m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>XGA161</td>
<td></td>
</tr>
<tr>
<td>XGA162</td>
<td></td>
</tr>
<tr>
<td>XGA179</td>
<td></td>
</tr>
<tr>
<td>XGA180</td>
<td></td>
</tr>
<tr>
<td>04AA215</td>
<td></td>
</tr>
<tr>
<td>04AA247</td>
<td></td>
</tr>
<tr>
<td>3940801</td>
<td></td>
</tr>
<tr>
<td>3940802</td>
<td></td>
</tr>
<tr>
<td>5680421</td>
<td></td>
</tr>
<tr>
<td>5680422</td>
<td></td>
</tr>
</tbody>
</table>

Table of 2000 L Scale Lots:

(taken from page 13 of Sponsor's Quality Overall Summary – Drug Product)

<table>
<thead>
<tr>
<th>Drug Substance Lot(s)</th>
<th>Formulated Drug Substance Lot</th>
<th>Formulated Drug Substance Lot</th>
<th>Drug Product Lot</th>
<th>Bioreactor Scale</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>XGA161</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>2000 L</td>
<td>Purified on 50 L, 50 L Equipment. Biochemical Comparability.</td>
</tr>
<tr>
<td>XGA162</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>2000 L</td>
<td>Purified on 50 L, 50 L Equipment. Biochemical Comparability.</td>
</tr>
<tr>
<td>04AA215</td>
<td>04AA215</td>
<td>N/A</td>
<td>N/A</td>
<td>2000 L</td>
<td>2000 L Purification Scale Biochemical Comparability.</td>
</tr>
<tr>
<td>04AA247</td>
<td>04AA247</td>
<td>N/A</td>
<td>2356399</td>
<td>2000 L</td>
<td>2000 L Purification Scale Biochemical Comparability.</td>
</tr>
<tr>
<td>3940801</td>
<td>3940801</td>
<td>N/A</td>
<td>3940801</td>
<td>2000 L</td>
<td>Clinical Trial Materials Stability Studies Process Validation.</td>
</tr>
<tr>
<td>3940802</td>
<td>3940802</td>
<td>N/A</td>
<td>3940802</td>
<td>2000 L</td>
<td>Clinical Trial Materials Stability Studies Process Validation.</td>
</tr>
<tr>
<td>5680421</td>
<td>5680421</td>
<td>N/A</td>
<td>4573352</td>
<td>2000 L</td>
<td>Clinical Trial Materials Stability Studies Process Validation.</td>
</tr>
<tr>
<td>5680422</td>
<td>5680422</td>
<td>N/A</td>
<td>5744693</td>
<td>2000 L</td>
<td>Clinical Trial Materials Stability Studies Process Validation.</td>
</tr>
</tbody>
</table>

Not Applicable. A Formulated Drug Substance, not produced with this material. 2000 L cell culture material.

All other 2000 L Drug Product lots were manufactured at Allergan, Landsting Information on the lyophilization of 2000 L material at 75 C is provided in Section 3.2.5.2.8.5.3.
Conclusion:

- The Sponsor proposed to market both the 160-L lots and the 2000-L lots of alglucosidase alfa. Only the 160-L lot was used in the clinical pivotal trial.
- Pharmacokinetic comparability between the 160-L and the 2000-L lots was studied in GAA knockout mice (Study 05-0414), and the results failed to demonstrate comparability between the 160-L and the 2000-L lots. The 2000-L lots had a 30% lower AUC than the 160-L lot.
- In a biodistribution study in GAA knockout mice (Study 05-0252), the 2000-L scale product had 28 to 81% higher liver uptake and 20 to 65% lower quadriceps uptake between 1 to 8 hours after dosing than the 160-L scale product.

4.2.10 Analytical Methods

Alglucosidase Assay: The recombinant human acid alpha-glucosidase (rhGAA) content was measured in human plasma samples, using 4-methylumbelliferyl alpha-D-glucopyranoside (4-MUG, ), as substrate. 4-MUG upon hydrolysis produces the fluorescent 4-methylumbelliferone (4-MU). The assay is quantified relative to the activity of an 8-point rhGAA standard curve prepared from a dilution series of reference rhGAA. The concentration of rhGAA in the sample was determined by interpolation from the standard curve and the data are expressed as units of concentration (ng/mL). Assay results were accepted if assay controls containing rhGAA read within the established range. The overall precision of interpolated enzyme mass values of the samples ranged from 9.7 to 15.2 % coefficient of variation. The linear range of the method was 2.5 to 500 ng/mL with a limit of quantification of 12.5 ng/mL.

GAA Activity in Skeletal Muscle Assay: Biopsies were obtained from the quadriceps muscle of patients at various time points for analysis. Frozen muscle biopsies were homogenized, sonicated, and then centrifuged. An aliquot of the resulting supernatant was used to assay GAA activity by the 4-MUG assay, which was carried out in skeletal muscle tissue homogenates using a method similar to that described for plasma samples. The use of 4-MUG provides a lower background from residual activity of neutral alpha-glucosidases than other substrates, such as maltose or glycogen; however, the assay does not discriminate between lysosomal and nonlysosomal GAA that is active at an acidic pH.

Biochemical Analysis of Glycogen Content in Skeletal Muscle: Glycogen content in skeletal muscle biopsies was measured using a 2-step biochemical procedure. After digesting the glycogen present in muscle tissues with amyloglucosidase, glucose was quantified with a Glucose Trinder Kit. Tissue samples were homogenized and then clarified by centrifugation and boiled. Control samples included buffer in place of the enzyme. The glucose produced was then measured by incubating the samples with Glucose Trinder Reagent. Absorbance was measured at 505 nm and quantified against a 7-point standard curve. Assay results were accepted if assay controls containing either glycogen or glucose read within the established range. An assay performance study suggests that differences of up to 10% may be attributed to assay variability. Therefore, a meaningful change in glycogen content was defined by Genzyme as a difference of > 20% (i.e., twice the variability in the assay) from Baseline. Glycogen content was considered to be stable if subsequent measurements were within 20% of the Baseline measurement.

Histomorphometric Analysis of Glycogen Content in Skeletal Muscle: The glycogen content in skeletal muscle was also quantified using a histomorphometric analytical procedure. The pathologist evaluating the tissue samples was blinded with respect to the identity of the
patients and the timing of the biopsy sample. MetaMorph® (version 4.6; Universal Imaging Corporation) is a proprietary image analysis system used by Genzyme to quantitatively measure glycogen content in the area of a 2-dimensional histological section of muscle tissue. One representative field from each slide was photographed with a digital camera and acquired with photo image capture software. Each digital image was formatted at a fixed pixel density using Adobe® PhotoShop® software. Each digital image was then opened using the MetaMorph Imaging Processing and Analysis software for histomorphometric analysis. MetaMorph, performed on periodic acid-Schiff (PAS)-stained tissue samples, selects and quantifies all purple pixels corresponding to accumulated glycogen. This value is the numerator. MetaMorph then selects and quantifies all color (both purple as well as the remaining light blue muscle tissue). This value is the denominator. A percentage is then calculated from these 2 values. This result represents the percent area of all tissue represented in a given image, which is occupied by the abnormal glycogen accumulation.

One representative field from each slide is evaluated. Up to 10 slides are analyzed per patient time point. The mean glycogen content and SD for each set are then calculated. This number represents the average glycogen content for each patient sample. The average and SD for the patient time point can then be calculated and used in comparing pre- and post-treatment values to determine the change in glycogen content. A meaningful change in glycogen content was defined by Genzyme as an estimate (mean glycogen content ± 1 SD) that did not overlap with the Baseline estimate. Stable glycogen content was defined as an estimate that overlapped with the Baseline estimate.

**Oligosaccharide Levels Assay:** Liquid chromatography-tandem mass spectrometry (LC-MS/MS) with stable isotope dilution was used to analyze oligosaccharides in plasma and urine. These measurements were performed at a central laboratory at Duke University Medical Center according to published and modified procedures.

### 4.3 Consult Review (Pharmacogenomics)

Consult Review below is from Pharmacogenomics Reviewer Emanuela Lacana.

**BLA 125141:** recombinant human acid alpha-glucosidase gene (Myozyme) for the treatment of infantile Pompe disease.

Pompe disease consists of genetically inherited defects in the acid alpha-glucosidase (GAA) gene. In the most severe form of the disease, with onset in the first months of life, GAA activity is absent or extremely low. This deficiency results in glycogen accumulation in the lysosome, ultimately leading to skeletal and cardiac muscle dysfunction. Over 70 mutations have been identified in GAA.

GAA is synthesized — Intermediate forms are generated by proteolytic cleavage, which is required to generate the fully activated enzyme.

_for review see Raben et. al 2002, Current Molecular Medicine, 2:145._

The Sponsor is proposing ____________

The Sponsor indicated the intent of using genetic and genomic studies for research purposes only in order to assess efficacy of Myozyme infusion in ERT.
Molecular genetic analysis in protocols AGLU01602-26 weeks and AGLU01702-52 weeks

Exploratory objectives (for research purposes only) included evaluation of the potential association of cross-reacting immunologic material (CRIM) status, angiotensin-converting enzyme (ACE) marker allele status, and GAA gene mutations with efficacy outcomes, as well as the potential effect of Myozyme on regulation of gene expression. The Sponsor submitted 2 studies, AGLU1602-26 week and AGLU1702-52 week. In both studies, the Sponsor indicated that exploratory analyses were not completed prior to BLA submission.

1) CRIM status: the 110 kDa precursor and the intermediate cleavage products have been defined by the Sponsor as Cross-Reactive Immunological Material. Presence of one or more CRIM band in Western Blots of patients' fibroblasts will be considered as CRIM positive. CRIM negativity is associated with a less favorable outcome, while the presence of one or more CRIM band indicates the presence of GAA. (However, presence of the protein does not necessarily indicate that the enzyme is active).

The Sponsor claims to have developed a panel of monoclonal antibodies that detect GAA intermediate forms. It is recommended that the Sponsor includes positive (fibroblasts from normal volunteers) and negative controls in WB, to ensure correct classification of patients as CRIM positive or negative.

2) ACE marker allele status: angiotensin-converting enzyme is encoded by the DCP1 gene. The DCP1 gene consists of two alleles, one with an insertion (I) of a 287 Alu fragment in intron 16, one without (D). The Sponsor claims an association between the D allele and prevalence of Type II muscle fibers (anaerobic metabolism) and I allele and prevalence of Type I muscle fibers (oxidative metabolism). The Sponsor is planning to assess the status of ACE marker by PCR on genomic DNA, using primer sequences published in the literature. The amplicons will be 190bp and 490bp for the D allele and the I allele, respectively. A comparison with known standards will be performed. The Sponsor reported that given the low number of patients with a D/D genotype (3 out of 15), no conclusions on efficacy outcome in relationship to genotype were drawn.

   a. It is recommended that the Sponsor specify the nature of the known standards. Plasmids containing the two allelic variant sequences would be best as positive controls. In addition, incorporation of a negative control is also recommended.

   b. A number of references in the literature show a relationship between the status of ACE allele and muscle endurance/performance capability, and support a role for ACE polymorphism in the regulation of human skeletal muscle strength. The Sponsor should clarify, however, what is the expected correlation with efficacy of Myozyme treatment and ACE status in the specific case of Pompe disease.

3) Genotyping of the GAA gene: DNA samples were prepared from blood of enrolled patients and their parents using standard techniques and sequenced. Several mutations were identified, both already characterized in the literature and novel.

4) Gene expression analysis: The purpose is to identify genes modulated in response to Myozyme treatment of patients with Pompe disease. The Sponsor will collect muscle
sample biopsies at baseline, week 12 and week 26 or 52 depending on the study. The Sponsor indicated that such analyses were not completed prior to BLA submission. Gene expression will be analyzed by Serial Analysis of Gene Expression (SAGE).

If the Sponsor intends to submit data to the BLA, a complete protocol for SAGE should be provided, which includes appropriate quality, negative and positive controls. In addition, it is recommended that genes identified by SAGE as being modulated by Myozyme treatment would be verified using a different analytical platform, such as QRT-PCR.

4.4 Attendance at Required Office Level OCPB Briefing

Attendance at the Required Office Level OCPB Briefing for BLA 125141/0 on February 28, 2006, from 3:00 to 4:30 PM was as follows:

1. Anil Rajpal
2. Hong Zhao
3. Shiew Mei Huang
4. Chandra Sahajwalla
5. John Hunt
6. John Lazor
7. Mehul Mehta
8. John Hyde
9. Anne Pariser
10. Frederick Mills
11. Hae Young Ahn
12. Ta-Chen Wu
13. Leslie Kenna
14. Jenny Zheng
15. Roshni Ramchandani
16. Sophia Abraham