

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: van der Ploeg AT, Clemens PR, Corzo D, et al. A randomized study of alglucosidase alfa in late-onset Pompe's disease. *N Engl J Med* 2010;362:1396-406.

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1. Methods

1.1 Study Design

This was a randomized, double-blind, placebo-controlled, multicenter study of the safety and efficacy of alglucosidase alfa in 90 patients with late-onset Pompe disease. Patients were pre-screened and provided written informed consent; they then underwent a full baseline screening evaluation (complete inclusion/exclusion criteria below). Qualifying patients were randomized 2:1 to receive 20 mg/kg alglucosidase alfa or placebo IV every other week (Supplementary Figure 1). The Pocock and Simon minimization algorithm¹ was used to balance baseline six-minute walk test (6MWT) distance walked (≥ 300 meters, < 300 meters) and % predicted forced vital capacity (FVC) upright score ($\geq 55\%$, $< 55\%$) across treatment arms within study sites. The minimization algorithm randomly assigned treatment assignments with probabilities adaptively weighted to minimize differences between the treatment groups across sites, baseline 6MWT and baseline FVC upright (% predicted). The stratification levels used for the baseline 6MWT and FVC upright were as follows:

(A) Baseline 6MWT ≤ 300 meters; (B) Baseline 6MWT > 300 meters

(A) Baseline FVC upright $\leq 55\%$ predicted; (B) Baseline FVC upright $> 55\%$ predicted

The choices of stratification cut points (i.e., 300 meters distance walked, 55% predicted FVC) were based on observed median values for the measurements from an appropriate set of patients in an observational study of late-onset Pompe disease.²

Patients were randomized by study personnel entering baseline information into an Interactive Voice Response System. The Interactive Voice Response System was linked to a centralized

computer that randomly assigned treatment via the minimization algorithm as implemented by proprietary and validated software developed by Covance, a contract research organization.

The Investigators, other study site personnel, the patients, and representatives of Genzyme Corporation, with the exception of Genzyme Clinical Pharmacy Research Services, remained blinded to treatment assignment for the duration of the study. Every effort was made to maintain the integrity of the blind.

Inclusion/Exclusion

Patients fulfilling the following criteria were eligible for inclusion in the study:

1. The patient provided signed, informed consent prior to performing any study-related procedures. Consent of a legally authorized guardian(s) was required for patients under 18 years of age. If the patient was under 18 years of age and could understand the written informed consent, signature was required from both the patient and the authorized guardian(s);
2. The patient had a diagnosis of Pompe disease based on deficient endogenous acid α -glucosidase (GAA) activity in cultured skin fibroblasts of $\leq 40\%$ of the normal mean of the testing laboratory and 2 GAA gene mutations;
3. The patient was ≥ 8 years of age at the time of enrollment;
4. The patient was able to ambulate 40 meters (approximately 130 feet) in 6 minutes on each test performed on 2 consecutive days (use of assistive devices such as a walker, cane, or crutches, was permitted);
5. The patient had an FVC of $\geq 30\%$ and $< 80\%$ predicted in the upright position³;

6. The patient had a postural drop in FVC (liters [L]) of at least 10% from the upright to the supine position $[(FVC \text{ supine (L)} - FVC \text{ upright (L)})/FVC \text{ upright (L)}] * 100\%$;
7. The patient had proximal muscle weakness in the lower limbs defined as unilateral QMT of the knee extensors $<80\%$ of the predicted value based on age, gender and body size⁴;
8. The patient was able to tolerate pulmonary function testing (PFT) and muscle testing in the supine position. (See exclusion criteria [1] and [2].);
9. The patient had testable muscle in bilateral knee flexors and knee extensors, and testable muscle in bilateral elbow flexors and elbow extensors. (Using QMT, a muscle was defined as “not testable” if the patient: 1) had a contracture >90 degrees that prevented being able to assume the standard testing position, 2) was unable to follow directions, 3) had significant pain with resistance to the motion, or 4) was so weak that force could not be generated against the testing strap);
10. The patient was able to provide reproducible muscle and pulmonary function test results (bilateral QMT measurements [% predicted] in knee extensors within 10% of the highest test value obtained from the same side of the body on 2 consecutive days and FVC measurements [in liters] within 10% of the highest test value obtained in the upright position on 2 consecutive days);
11. The patient (and patient’s legal guardian if patient was <18 years of age) had the ability to comply with the clinical protocol;
12. A female patient of childbearing potential was required to have a negative pregnancy test (urine beta-human chorionic gonadotropin [β -hCG]) at Baseline. Note: All female patients of childbearing potential and sexually mature males were required to use a

medically accepted method of contraception throughout the study.

Patients fulfilling any of the following criteria were excluded from study participation:

1. The patient required the use of invasive ventilatory support. (Invasive ventilation was defined as any form of ventilatory support applied with the use of an endotracheal tube.);
2. The patient required the use of noninvasive ventilatory support while awake and in an upright position. (Noninvasive ventilation was defined as any form of ventilatory support applied without the use of an endotracheal tube. For example, patients receiving positive-pressure ventilation support through a facemask or nose piece were considered as ventilated through noninvasive methods.);
3. The patient had received enzyme replacement therapy with GAA from any source;
4. The patient had used an investigational product within 30 days prior to study enrollment, or was currently enrolled in another study that involved clinical evaluations, unless prior approval was given by Genzyme;
5. The patient had a major congenital anomaly, medical condition, serious intercurrent illness, or other extenuating circumstance that, in the opinion of the Investigator, may have significantly interfered with study compliance, including all prescribed evaluations and follow-up activities.

1.2 Adaptive Design Strategy

An adaptive two-stage study design was implemented (under a protocol amendment) in order to ensure that the trial terminated with a robust finding, either of efficacy or lack of efficacy as

demonstrated by change in 6MWT, without compromising the type I error rate. The adaptive design was developed in consultation with an external independent statistical center and in accordance with the comments and cautions on sponsor access to interim data expressed in the guidance ‘On the Establishment and Operation of Clinical Trial Data Monitoring Committees’ (March 2006). The external independent statistical center (Cytel, Inc.) and the Data Safety Monitoring Board reviewed and approved the adaptive design charter prior to implementation. While the independent statistical center and the Data Safety Monitoring Board were unblinded to treatment assignment during the course of the interim analysis and review, the study sponsor did not have access to the results of the adaptive design analysis until the conclusion of the trial (i.e., database lock and treatment code unblinding for the final analysis).

The adaptive two-stage design for this trial involved performing an interim analysis that included fitting the primary efficacy model and estimating the amount of statistical information that was currently available. The results of the model fitting were used to estimate the duration of patient follow-up required to achieve a pre-specified level of statistical information relevant to the primary efficacy analysis and potentially adapting the duration of the trial based on this estimate. Because this procedure only used the interim estimate of the statistical information (proportional to the standard error of the estimated treatment effect), no adjustment to the Type I error rate was needed.

The planned model for the primary efficacy analysis was a linear mixed effects model with inference based on the difference of the slopes of change in 6MWT between alglucosidase alfa and placebo. In the interim analysis, the model was fit to the patients in the full analysis

population that comprised all patients randomized. For the purpose of the adaptive design, complete information was prospectively specified as $I_{\max} = 0.747$, which was based on the following parameterization: treatment effect of 3.75 meters/month, 90% power and a 5% significance level.

The adaptive design analysis was performed in the middle of December 2006, at which time the last patient enrolled and continuing in the study had completed 38 weeks of post-treatment follow-up and no patient had completed the planned 52 weeks of post-treatment follow-up. The independent statistical center applied pre-specified rules to evaluate whether, based on the adaptive trial strategy, the trial was to be continued for an additional 3 (until approximately March 2007), 6 (until approximately June 2007), or 9 months (until approximately September 2007) (i.e., until the last patient completed Week 52, Week 64, or Week 78 of follow-up, respectively), on the basis of the estimated time at which the full amount of statistical information targeted at the start of the trial was expected to be obtained. The pre-specified rules that were developed with respect to the primary efficacy endpoint were as follows:

- If the full information had arrived at the time of the adaptive design analysis, then the recommendation to terminate the trial in March 2007 was to be made to the Data Safety Monitoring Board.
- If the full information was expected to arrive between January 2007 and March 2007, then the recommendation to terminate the trial in March 2007 was to be made to the Data Safety Monitoring Board.

- If the full information was expected to arrive between March 2007 and June 2007, then the recommendation to terminate the trial in June 2007 was to be made to the Data Safety Monitoring Board.
- If the full information was expected to arrive between June 2007 and September 2007, then the recommendation to terminate the trial in September 2007 was to be made to the Data Safety Monitoring Board.
- If the full information was not expected to arrive until after September 2007, then the recommendation to terminate the trial in September 2007 was to be made to the Data Safety Monitoring Board.

The independent statistical center was responsible for communicating the results of the adaptive design analysis to the Data Safety Monitoring Board.

Based on this interim analysis, the Data Safety Monitoring Board recommended that the study duration be extended to 78 weeks for all patients. The treatment regimen was extended to 78 weeks. All patients were maintained on the same treatment regimen they had been initially randomized to receive. The study sponsor and investigators had no access to the interim results until study conclusion.

1.3 Subgroup Analyses

Treatment effects were also analyzed within baseline factors using predefined subgroups. The predefined subgroups included gender (male / female), age at first infusion (<45 years / \geq 45

years), baseline 6MWT randomization strata (<300 meters / \geq 300 meters), baseline FVC randomization strata (<55% / \geq 55%), baseline walking device use (yes / no), baseline noninvasive ventilator status (ventilated / not ventilated), time since symptom onset (<15 years / \geq 15 years) and baseline GAA activity (<9.8% normal / \geq 9.8% normal). A post hoc baseline factor was age at Pompe symptom onset. The subgroups for this factor were defined by the observed baseline mean. ANCOVA models were used with baseline, baseline factor, treatment and treatment-by-baseline factor interaction. The ANCOVA model used last observation carried forward. The least-square means estimates and associated 95% confidence interval are reported in Supplementary Figure 1 for all pre-specified factors as well as for age at Pompe symptom onset.

1.4 Sensitivity Analysis of 6MWT and FVC (% Predicted)

In order to assess the robustness of the primary efficacy endpoint results, a sensitivity analysis was performed using alternative statistical methods. The results of these tests are contained in Supplementary Table 1 and substantiate the conclusion that the estimated treatment effects are statistically significant. Results are presented for 6MWT and FVC % predicted.

The sensitivity analysis included a mixed model for repeated measures (MMRM) that effectively accounts for missing data without explicit imputation such as LOCF.⁵ This model uses all of the available repeated efficacy measurements. The covariates for this model were baseline, factor variables for site, time, treatment, time-by-treatment interaction and baseline-by-time interaction. The treatment effect was estimated as the difference in the change from baseline to Week 78.

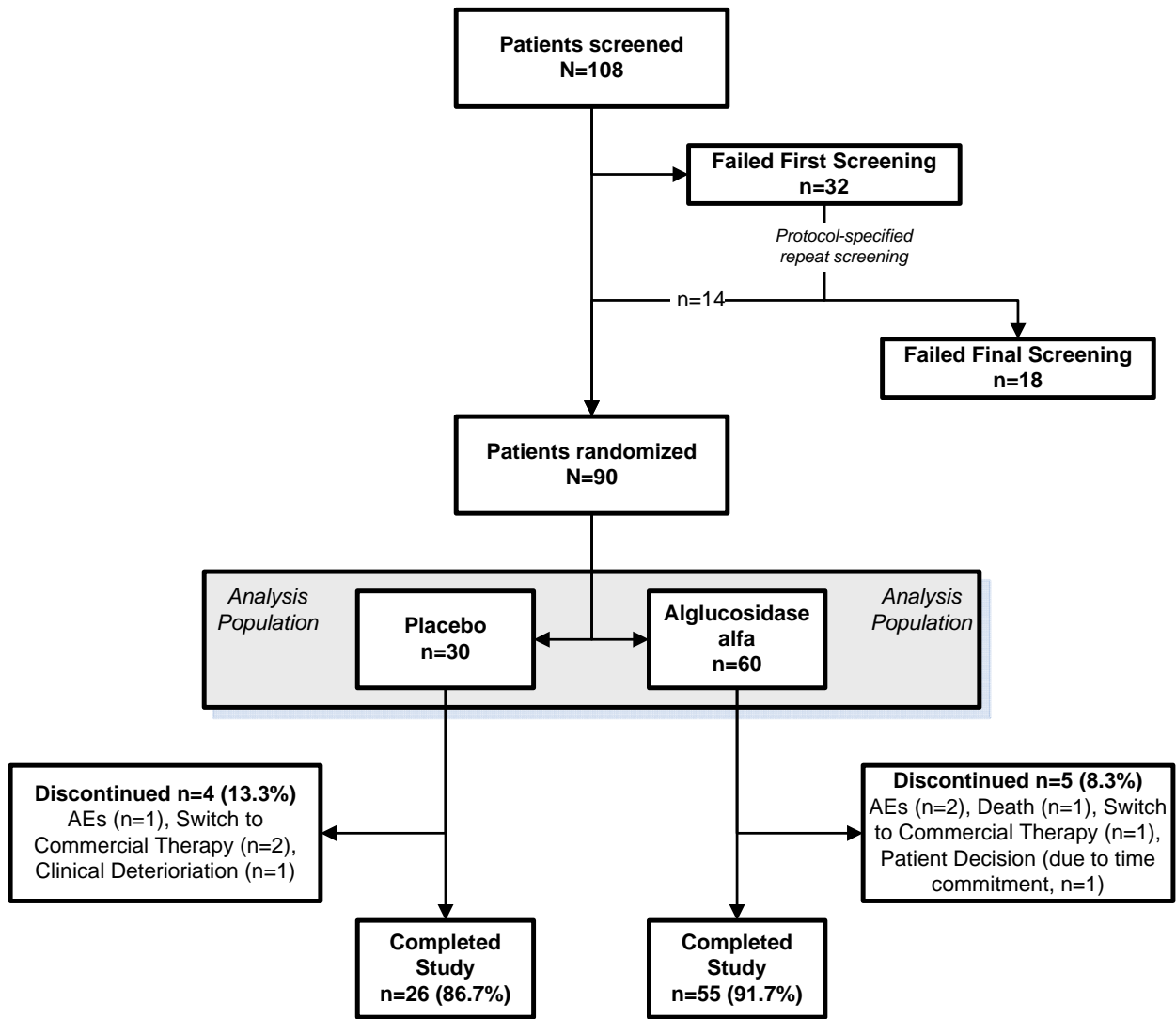
Another method used in the sensitivity analysis was the nonparametric Wilcoxon-Mann-Whitney test. This test is distribution-free in that it does not make strong assumption regarding the underlying 6MWT or % predicted FVC distribution. The WMW test was stratified by randomization strata. These analyses included several approaches to imputing the missing data for patients that discontinued early from the study or otherwise had missing data at week 78.

The following methods were used:

- Last observation carried forward (LOCF)
- Multiple imputations: The regression model approach was used to create the multiple imputations and this was followed by application of the WMW test. This approach has been described in detail.⁶
- Worst-rank analysis: For patients with missing week 78 data, a value was assigned that gives them the worst ranking in the WMW test. This approach has been described in detail.⁷

2. Supplementary Figures

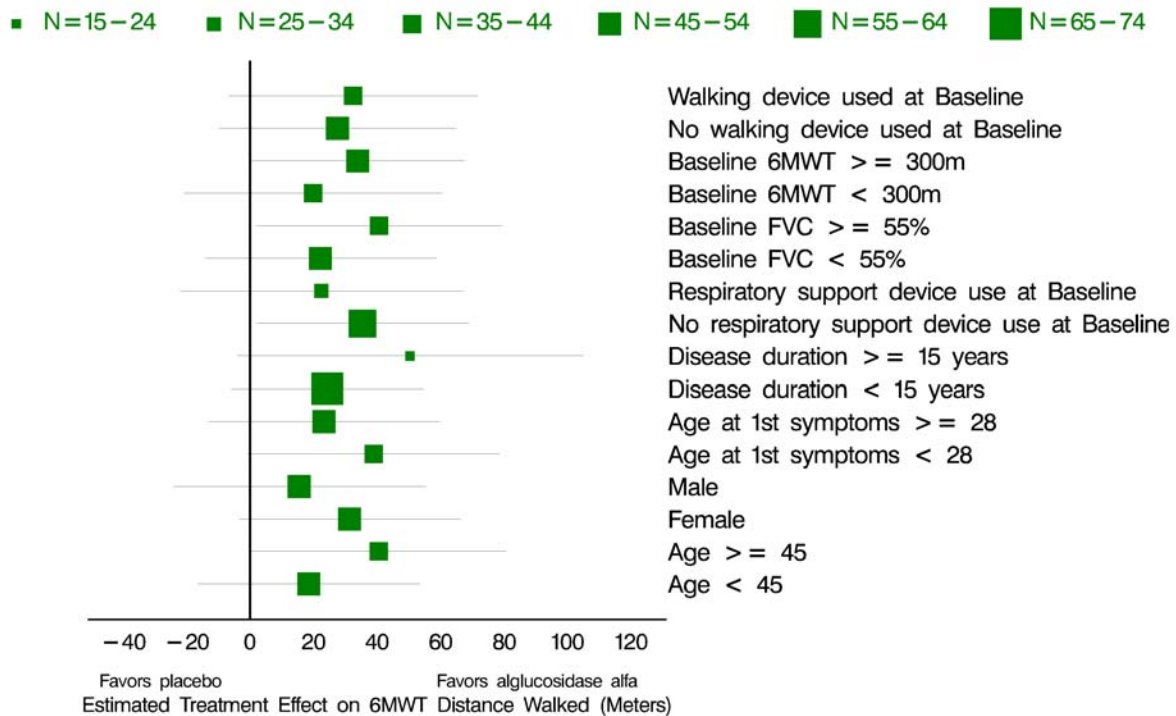
2.1 Supplementary Figure 1. Patient Flow



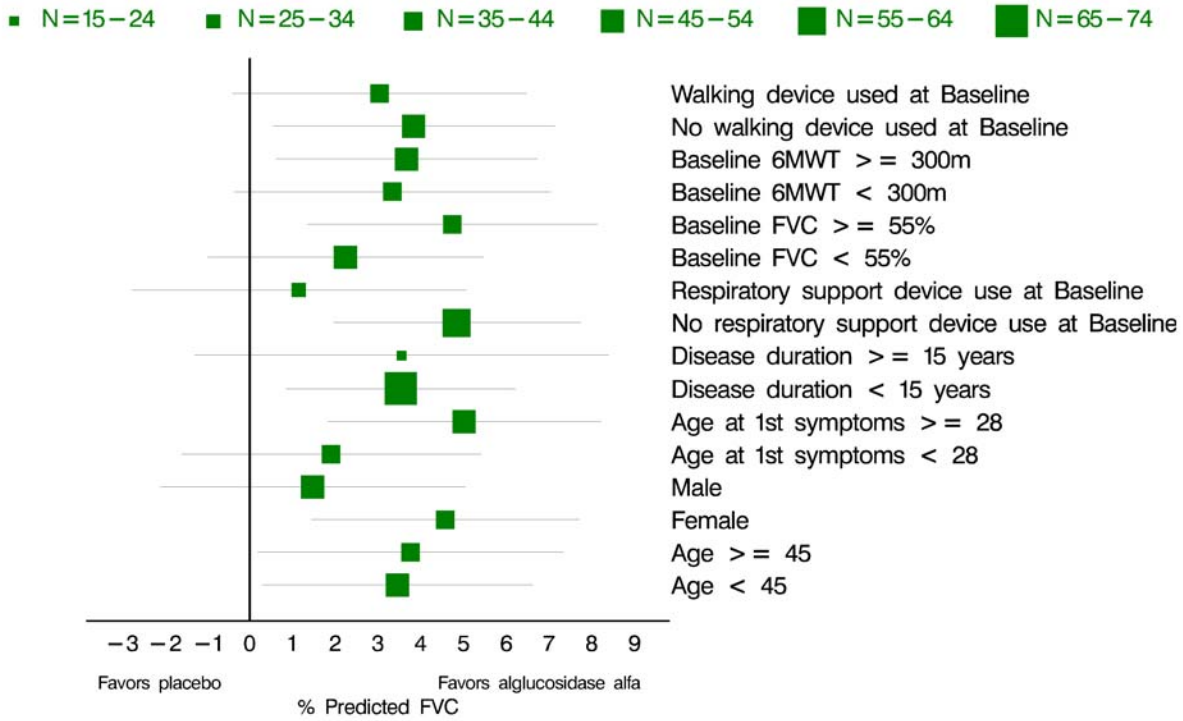
2.2 Supplementary Figure 2. Treatment Effect of Alglucosidase alfa on Walking Distance

(A) and Pulmonary Function (B) across Various Patient Subgroups. Squares indicate the estimated difference in 6MWT distance walked (a) or % predicted FVC (b) between the alglucosidase alfa treatment group and the placebo group for various patient subgroups. The horizontal line is the 95% confidence interval. Estimates to the right of the vertical line (greater than 0) favor alglucosidase alfa. The squares are scaled to the sample size within each subgroup, as shown in the legend.

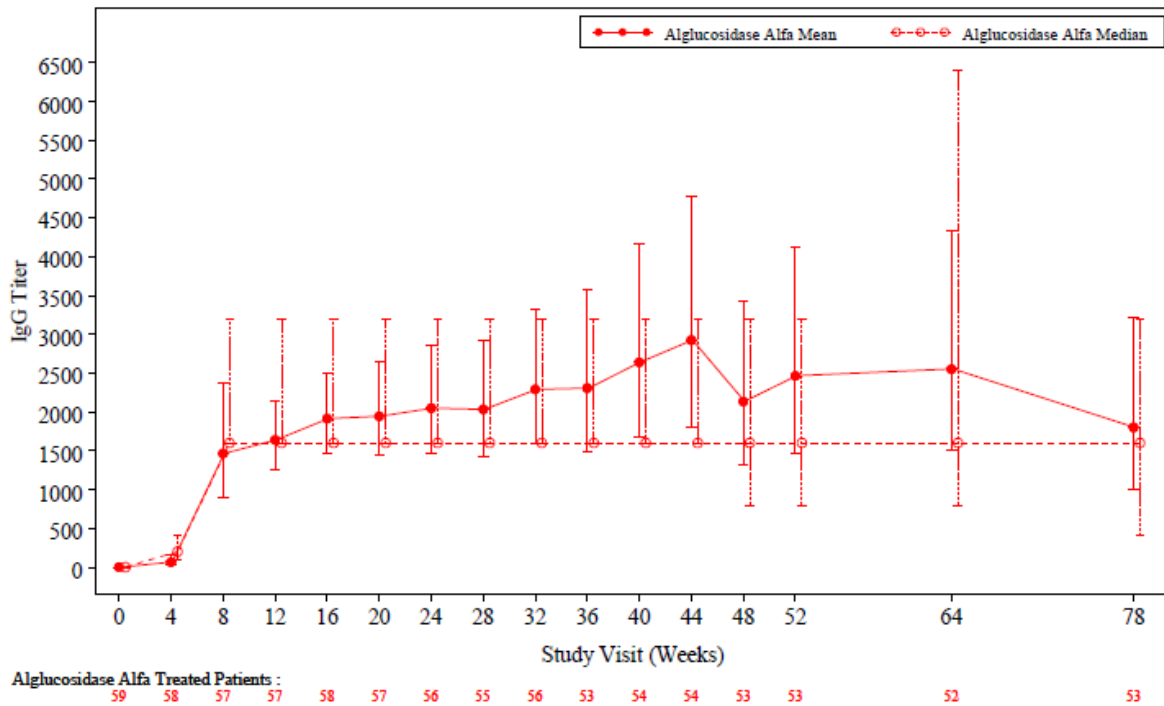
A.



B.



2.3 Supplementary Figure 3. Geometric Mean and Median IgG Titer Values over Time for All Alglucosidase alfa-treated Patients.



Note: Antibody titers observed in a cross-reactive immunologic material-negative (CRIM-negative) population studied by Kishnani et al. ranged from 0 to 1/1,638,400.⁸ By contrast, antibody titers in the current patient population ranged from 1/200 to 1/819,200. It is important to note that patients with late-onset Pompe disease are CRIM-positive, meaning they have some residual GAA protein. This probably explains why patients with late-onset Pompe disease do not tend to mount the high and sustained antibody response seen in CRIM-negative individuals, who have little to no residual protein.

3. Supplementary Tables

3.1 Supplementary Table 1: Results of Sensitivity Analyses Comparing Six-minute Walk Test and Percent Predicted FVC Change from Baseline to Week 78 between Alglucosidase alfa- and Placebo-treated Patients

Endpoint	Method	Imputation Technique	Statistics	Results
Six-minute Walk Test distance walked, meters	Mixed model for repeated measures	None	Estimate (95% CI)	32.0 m (7.2, 56.8)
			p-value	0.01
	Nonparametric Wilcoxon-Mann-Whitney	LOCF	p-value	0.029
		Multiple imputation	p-value	0.028
		Worst-rank	p-value	0.04
FVC (% predicted)	Mixed model for repeated measures	None	Estimate (95% CI)	3.6 % (1.4, 5.8)
			p-value	0.001
	Nonparametric Wilcoxon-Mann-Whitney	LOCF	p-value	0.003
		Multiple imputation	p-value	0.002
		Worst-rank	p-value	0.002

3.2 Supplementary Table 2: Summary of Treatment-emergent Adverse Events Considered Related to Treatment Occurring in at Least 5% of Patients by Treatment Group

System Organ Class Preferred Term	Number of Patients Receiving Alglucosidase alfa	Number of Patients Receiving Placebo
	n (%)	n (%)
Any Adverse Events	32 (53.3)	17 (56.7)
General disorders and administration site conditions	15 (25.0)	7 (23.3)
Fatigue	3 (5.0)	4 (13.3)
Chest discomfort	4 (6.7)	1 (3.3)
Asthenia	0	2 (6.7)
Nervous system disorders	10 (16.7)	8 (26.7)
Headache	5 (8.3)	6 (20.0)
Dizziness	4 (6.7)	2 (6.7)
Skin and subcutaneous tissue disorders	13 (21.7)	4 (13.3)
Urticaria	5 (8.3)	0
Hyperhidrosis	5 (8.3)	0
Gastrointestinal disorders	9 (15.0)	4 (13.3)
Nausea	5 (8.3)	3 (10.0)
Vomiting	3 (5.0)	0
Musculoskeletal and connective tissue disorders	8 (13.3)	2 (6.7)
Muscle twitching	4 (6.7)	1 (3.3)

Myalgia	3 (5.0)	1 (3.3)
Eye disorders	6 (10.0)	2 (6.7)
Cataract	4 (6.7)	1 (3.3)
Ear and labyrinth disorders	4 (6.7)	2 (6.7)
Hypoacusis	2 (3.3)	2 (6.7)
Vascular disorders	4 (6.7)	2 (6.7)
Flushing	3 (5.0)	0
Investigations	4 (6.7)	0
Blood pressure increased	3 (5.0)	0

3.3 Supplementary Table 3: Summary of Infusion-associated Reactions Occurring in at Least 5% of Patients by Treatment Group

System Organ Class Preferred Term	Number of Patients Receiving Alglucosidase alfa n (%)	Number of Patients Receiving Placebo n (%)
	Any Infusion-associated Reactions	17 (28.3)
Nervous system disorders	9 (15.0)	6 (20.0)
Headache	5 (8.3)	5 (16.7)
Dizziness	4 (6.7)	2 (6.7)
General disorders and administration site conditions	10 (16.7)	2 (6.7)
Chest discomfort	4 (6.7)	0
Gastrointestinal disorders	8 (13.3)	3 (10.0)
Nausea	5 (8.3)	3 (10.0)
Vomiting	3 (5.0)	0
Skin and subcutaneous tissue disorders	10 (16.7)	0
Urticaria	5 (8.3)	0
Hyperhidrosis	3 (5.0)	0
Vascular disorders	3 (5.0)	1 (3.3)
Flushing	3 (5.0)	0
Investigations	3 (5.0)	0
Blood pressure increased	3 (5.0)	0

3.4 Supplementary Table 4. Summary of Safety and Efficacy in Alglucosidase alfa-treated Patients by Peak IgG Titer Quartiles

Parameter	Peak IgG Titer Category for Alglucosidase alfa Patients Who Seroconverted (N = 59)			
	Quartile 1 200-1600	Quartile 2 3200-3200	Quartile 3 6400-12800	Quartile 4 25600-819200
Number of Patients n (%)	17 (28.8)	12 (20.3)	16 (27.1)	14 (23.7)
Number of Patients with any AE n (%)	17 (100.0)	12 (100.0)	16 (100.0)	14 (100.0)
Number of Patients with any SAE n (%)	5 (29.4)	2 (16.7)	4 (25.0)	1 (7.1)
Number of Patients with any IAR n (%)	6 (35.3)	2 (16.7)	5 (31.3)	3 (21.4)
6MWT change in meters walked from Baseline to last observation	6.1±53.67 median 5.0	16.0±24.98 median 9.0	34.8±76.60 median 16.5	49.1±79.91 median 19.5
FVC change in % predicted from Baseline to last observation	0.8±5.68 median 0.0	1.8±5.29 median 3.0	1.5±5.73 median 0.5	1.1±5.95 median 0.0

3.5 Supplementary Table 5: Inhibitory and IgG Antibodies for Patients Receiving Alglucosidase alfa Who Improved ≥ 25 M Compared with the Rest of Treated Patients

	Patients who improved ≥ 25 M N=27	Patients who did not improve ≥ 25 M N=30	p-value*
Inhibitory antibody status:			
Positive, N (%)	7 (26)	10 (33)	0.58
Peak IgG titer:			
N	27	30	
Median	6400	4800	0.85
Min, Max	1600, 204800	200, 819200	
IgG titer at Week 24:			
N	26	29	
Median	1600	1600	0.63
Min, Max	400, 25600	200, 51200	

* Inhibitory antibody status was tested using Fisher's exact test. IgG titer data are tested using Wilcoxon test.

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