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Safety, tolerability, pharmacokinetics, pharmacodynamics, and exploratory efficacy of the novel enzyme replacement therapy avalglucosidase alfa (neoGAA) in treatment-naïve and alglucosidase alfa-treated patients with late-onset Pompe disease: A phase 1, open-label, multicenter, multinational, ascending dose study

Loren D.M. Pena[a,*], Richard J. Barohn[b], Barry J. Byrne[c], Claude Desnuelle[d], Ozlem Goker-Alpan[e], Shafeeq Ladha[f], Pascal Laforêt[g], Karl Eugen Mengel[h], Alan Pestronk[i], Jean Pouget[j], Benedikt Schoser[k], Volker Straub[l], Jaya Trivedi[m], Philip Van Damme[n], John Vissing[o], Peter Young[p], Katherine Kacena[q], Raheel Shafi[q], Beth L. Thurberg[q], Kerry Culm-Merdek[q], Ans T. van der Ploeg[r], On behalf of the NEO1 Investigator Group

[a] *Duke University Medical Center, Durham, NC, USA* [b] *University of Kansas Medical Center, Kansas City, KS, USA* [c] *University of Florida, Gainesville, FL, USA* [d] *University Hospital of Nice, Côte d'Azur University, Nice, France* [e] *O and O Alpan LLC, Fairfax, VA, USA* [f] *Barrow Neurological Institute, Phoenix, AZ, USA* [g] *Centre de Référence des Maladies Neuromusculaires Nord/Est/Ile de France Service de Neurologie, Hôpital Raymond-Poincaré, Garches, AP-HP and INSERM U1179, Université Versailles Saint-Quentin-en-Yvelines, Montigny-le-Bretonneux, France* [h] *Villa Metabolica, Centre for Pediatric and Adolescent Medicine, University Medical Center, Mainz, Germany* [i] *Washington University School of Medicine, St Louis, MO, USA* [j] *CHU Timone APHM, Marseille, France* [k] *Friedrich-Baur-Institut, Department of Neurology Klinikum München, München, Germany* [l] *Newcastle University John Walton Muscular Dystrophy Research Centre, Newcastle Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK* [m] *University of Texas Southwestern Medical Center, Dallas, TX, USA* [n] *KU Leuven (Catholic University of Leuven), Department of Neurosciences, VIB - Center for Brain & Disease Research, and University Hospitals Leuven, Department of Neurology, Leuven, Belgium* [o] *Copenhagen Neuromuscular Center, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark* [p] *Department of Sleep Medicine and Neuromuscular Disorders, Universitätsklinikum Münster, Münster, Germany* [q] *Sanofi Genzyme, Cambridge, MA, USA* [r] *Erasmus Medical Center, Pompe Center, Rotterdam, The Netherlands*

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Abstract

This multicenter/multinational, open-label, ascending-dose study (NCT01898364) evaluated safety, tolerability, pharmacokinetics, pharmacodynamics, and exploratory efficacy of repeat-dose avalglucosidase alfa (neoGAA), a second-generation, recombinant acid α-glucosidase replacement therapy, in late-onset Pompe disease (LOPD). Patients ≥18 years, alglucosidase alfa naïve (Naïve) or previously receiving alglucosidase alfa for ≥9 months (Switch), with baseline FVC ≥50% predicted and independently ambulatory, received every-other-week avalglucosidase alfa 5, 10, or 20 mg/kg over 24 weeks. 9/10 Naïve and 12/14 Switch patients completed the study. Avalglucosidase alfa was well-tolerated; no deaths/life-threatening serious adverse events (SAEs). One Naïve patient withdrew for study drug-related SAEs (respiratory distress/chest discomfort). Infusion-associated reactions (IARs) affected 8 patients. Most treatment-emergent AEs/IARs were non-serious with mild-to-moderate intensity. At screening, 5 Switch patients tested positive for anti-avalglucosidase alfa antibodies; on-treatment, 2 Switch and 9 Naïve patients seroconverted. Post-infusion, avalglucosidase alfa plasma concentrations declined monoexponentially ($t_{1/2z}$∼1.0 h). AUC was 5–6 × higher in the 20 vs 5 mg/kg group. Pharmacokinetics were similar between Switch and Naïve groups and over time. Baseline quadriceps muscle glycogen was low (∼6%) in most patients, generally remaining unchanged thereafter. Exploratory efficacy parameters (pulmonary function/functional capacity) generally remained stable or improved. Avalglucosidase alfa's well-tolerated safety profile and exploratory efficacy results support further avalglucosidase alfa development.

Keywords: Alglucosidase alfa; Enzyme replacement therapy; Glycogen storage disease type II; Lysosomal acid α-glucosidase (GAA) deficiency; Avalglucosidase alfa (neoGAA); Late-onset Pompe disease (LOPD).

* Corresponding author:
E-mail address: Loren.Pena@cchmc.org (L.D.M. Pena).

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1. Introduction

Pompe disease, also known as glycogen storage disease type II or acid maltase deficiency, is a progressive, rare autosomal recessive disorder caused by mutations in the gene that encodes acid α-glucosidase (GAA). Deficiency of lysosomal GAA, an enzyme required for lysosomal glycogen degradation, results in an accumulation of lysosomal and eventually cytoplasmic glycogen [1–3]. Accumulation of glycogen in the lysosome results in lysosomal swelling and rupture [4]. Pompe disease exists as a spectrum of phenotypes, and is often classified by age and symptoms at onset. Classical infantile Pompe disease (IOPD) presents shortly after birth and is characterized by prominent cardiomyopathy, progressive generalized hypotonia, and rapid progression; patients generally die within the first year when untreated. When Pompe disease has a more gradual onset (late-onset Pompe disease; LOPD), it presents as a proximal myopathy with respiratory muscle involvement including the diaphragm, but without cardiomyopathy. In these children and adults, the disease course is more variable [5]. Onset of clinical symptoms typically may occur from any time after the first year of life to as late as the eighth decade [6,7]; a small subset of patients present without cardiomyopathy at <1 year of age [5,6]. The vast majority of patients present during adulthood. Pompe disease incidence varies depending on patients' ethnicity, geographical region, and between the different phenotypes [1].

Muscle pathology in LOPD is characterized by progressive muscle damage starting with intralysosomal glycogen to accumulation of storage material [8], autophagic build-up [9], loss of contractile structure [10], muscle atrophy and replacement by fat [11–13], and concomitant progressive loss of function [14]. This process may already start in early life [15] and clinically diagnosed symptomatic patients' health and functional status may already be severely impaired at presentation [15–17]. For symptomatic patients with LOPD, the risk of wheelchair use increases by 13% and the risk of ventilator dependency increases by 8% for each additional post-diagnosis year without treatment [18]. Patients requiring both a wheelchair and respiratory support have higher mortality than those requiring neither (5-year survival: 74% vs 95%, respectively; $p<0.002$) [19].

Response to GAA replacement is determined by the severity of damage at treatment initiation and extent of lysosomal glycogen accumulation [15,20,21], and by the magnitude of muscle GAA uptake achieved on therapy [22,23]. Alglucosidase alfa is approved worldwide [24,25] for Pompe disease treatment. Initial clinical trials in IOPD patients demonstrated that alglucosidase alfa treatment prolonged both overall and invasive ventilation-free survival, as well as generally improved cardiomyopathy, motor skills, and independent functional capacity [26,27]. The Late-Onset Treatment Study (LOTS) [28,29], conducted in children (aged ≥8 years) and adults with Pompe disease, demonstrated that alglucosidase alfa stabilized respiratory function and improved walking distance, and supported the approval of alglucosidase alfa therapy for the whole spectrum of Pompe disease.

Alglucosidase alfa clears glycogen storage in cardiac muscle more effectively than in skeletal muscle [20,27], which may, in part, reflect tissue differences in cation-independent mannose 6-phosphate (CIM6P)/insulin growth factor II (IGFII) receptor expression and enzyme uptake [26–32]. Also, most LOPD patients, who typically have more residual GAA activity than patients with the infantile form, are generally spared cardiac involvement, indicating that cardiac muscle is likely to require less GAA activity to prevent and remediate glycogen storage [2]. The cell-surface CIM6P/IGFII receptor mediates cellular uptake of exogenous GAA and targets it to the lysosomal compartment [33]. Thus, M6P levels and exposure enable enzyme uptake and internalization, and increasing M6P on the recombinant enzyme may increase achieved skeletal muscle uptake [34]. Avalglucosidase alfa (neoGAA) is a second-generation, glycoengineered recombinant GAA replacement therapy with increased bis-M6P levels on the molecule in order to increase receptor-mediated uptake (Fig. 1).

Preclinical studies in a Pompe disease mouse model have shown that compared with alglucosidase alfa, avalglucosidase alfa has a 1000-fold higher binding affinity to M6P receptors [22,23] and greater glycogen clearance from muscle on a mg alglucosidase alfa/mg avalglucosidase alfa basis, i.e., equivalent clearance to rhGAA at one-fifth the dose [22]. In *GAA* knockout mice, 4 mg/kg is the minimum pharmacologically active dose for cardiac and 12 mg/kg for skeletal muscle [35]. The first dose of avalglucosidase alfa administered to patients in our study was 5 mg/kg actual body weight, which was cumulatively based upon nonclinical safety information, the maximum recommended starting dose per the Food and Drug Administration guidance [36], International Conference on Harmonization M3 guidance [37], and the observed potency and pharmacologically active dose of avalglucosidase alfa in all target tissues from multiple nonclinical studies in mice. Avalglucosidase alfa doses were planned to be administered in an ascending manner, and for each dose escalation the increment was 2-fold, and was supported by the monitoring of potential safety signals. This escalation strategy took into consideration the pharmacologically active dose of 12 mg/kg in skeletal muscle from preclinical studies [35].

The objectives of this open-label, ascending-dose study (NEO1; ClinicalTrials.gov identifier: NCT01898364) were to evaluate the safety and tolerability of avalglucosidase alfa, characterize its pharmacodynamic and pharmacokinetic profiles, and assess exploratory efficacy endpoints following repeated dose administrations in adults with Pompe disease, who were either alglucosidase alfa-naïve (Naïve Group) or had received alglucosidase alfa for ≥9 months (Switch Group). We emphasize that this study was, by design, a phase 1 study focusing on safety and enrolling a small patient cohort; for this reason the efficacy assessments were designated by protocol as exploratory.

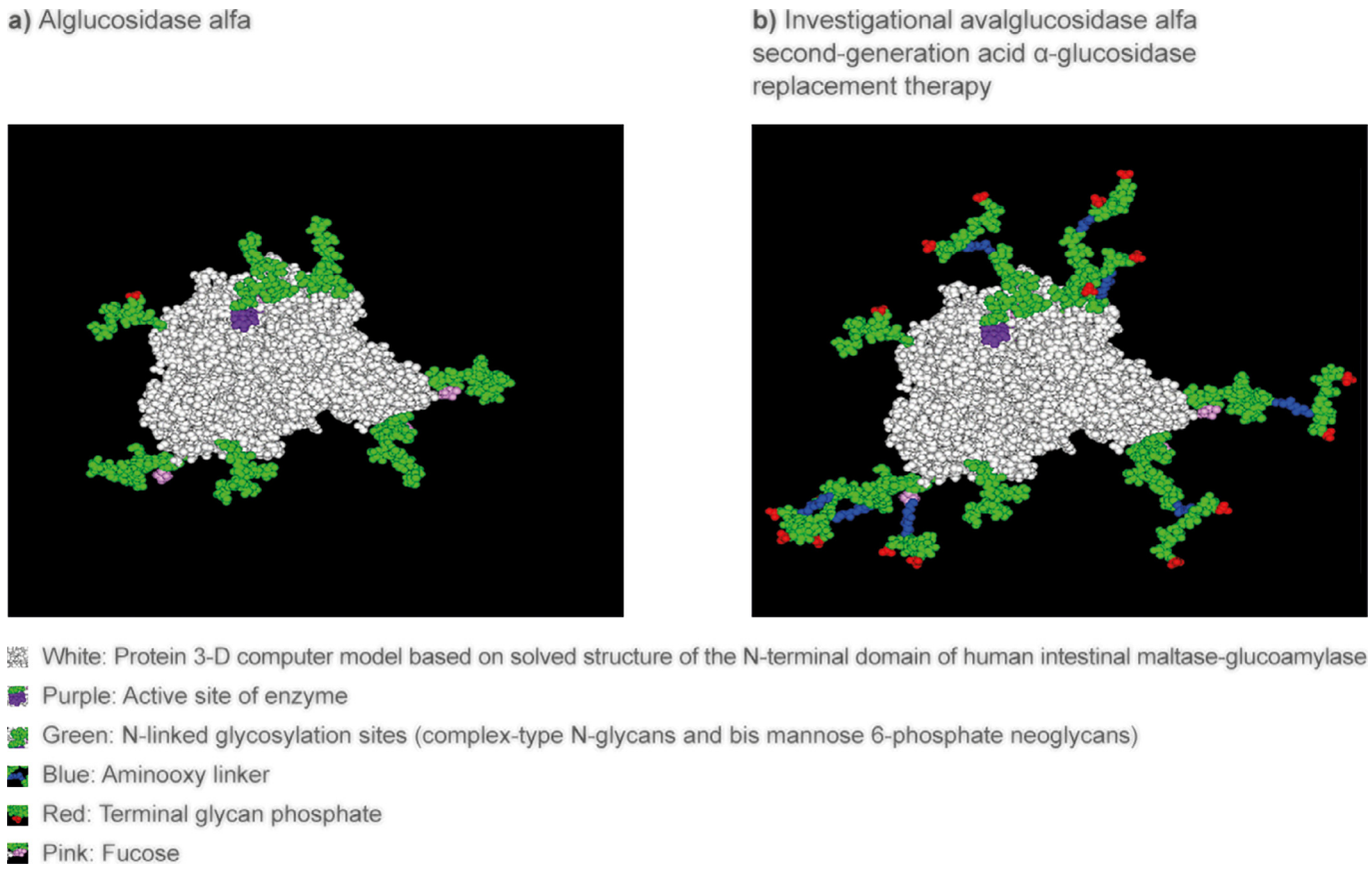

Fig. 1. Structures of (a) Alglucosidase alfa and (b) Investigational avalglucosidase alfa second-generation acid α-glucosidase replacement therapy. Increased bis-mannose 6-phosphate (M6P) levels on avalglucosidase alfa favor uptake by cation-independent M6P receptors. Fig. 1b Reprinted (adapted) with permission from Zhou Q, et al. *Bioconjugate Chem* 2011;22:741–751. Copyright 2011 American Chemical Society.

2. Patients and methods

2.1. Participants and study design

This was a multicenter, multinational, open-label, ascending dose study, with every-other-week intravenous (IV) infusions of avalglucosidase alfa; the study design is presented in Fig. 2.

Eligible patients were male or female, aged ≥18 years, with confirmed GAA enzyme deficiency from any tissue source and/or two confirmed pathogenic *GAA* gene variants, and without known cardiac hypertrophy. Samples for *GAA* genotyping were taken at baseline, if historical samples were not available. Patients had to be able to walk 50 m without stopping or using an assistive device and had to have an upright forced vital capacity (FVC) of ≥50% predicted according to Hankinson et al. [38]. Fertile women had to test negative for pregnancy at baseline and, if sexually active, use two acceptably effective contraception methods during treatment.

Patients were excluded if they were wheelchair-dependent, required invasive ventilation, were participating in another clinical study using investigational treatments, or were, in the opinion of the investigator, unable to adhere to study requirements. Patients were also excluded if they had clinically significant organic disease (apart from Pompe disease symptoms), e.g., cardiovascular, hepatic, pulmonary, neurologic, or renal disease, or had other medical conditions, serious intercurrent illness, or extenuating circumstance that, in the opinion of the investigator, precluded participation in the study or potentially decreased survival. Patients with MRI contraindications were also excluded. Conditions suggesting high risk for allergy to avalglucosidase alfa, e.g., previous moderate-to-severe anaphylactic reaction to alglucosidase alfa, immunoglobulin (Ig) E antibodies, and/or a history of sustained high IgG antibody titers to alglucosidase alfa, were exclusionary.

Patients were screened within 90 days before study inclusion and treatment initiation. For the Naïve Group, skeletal muscle MRI and biopsy and all baseline efficacy assessments were performed ≤21 days before avalglucosidase alfa initiation, and for the Switch Group, these were performed after the last alglucosidase alfa administration and ≤21 days before avalglucosidase alfa initiation.

Eligible patients, either naïve to alglucosidase alfa therapy (Naïve Group) or previously treated with alglucosidase alfa for ≥9 months (Switch Group), received every-other-week avalglucosidase alfa IV infusions at 5, 10, or 20 mg/kg actual body weight, totaling 13 infusions over 24 weeks. Each infusion was administered stepwise, beginning at a slow

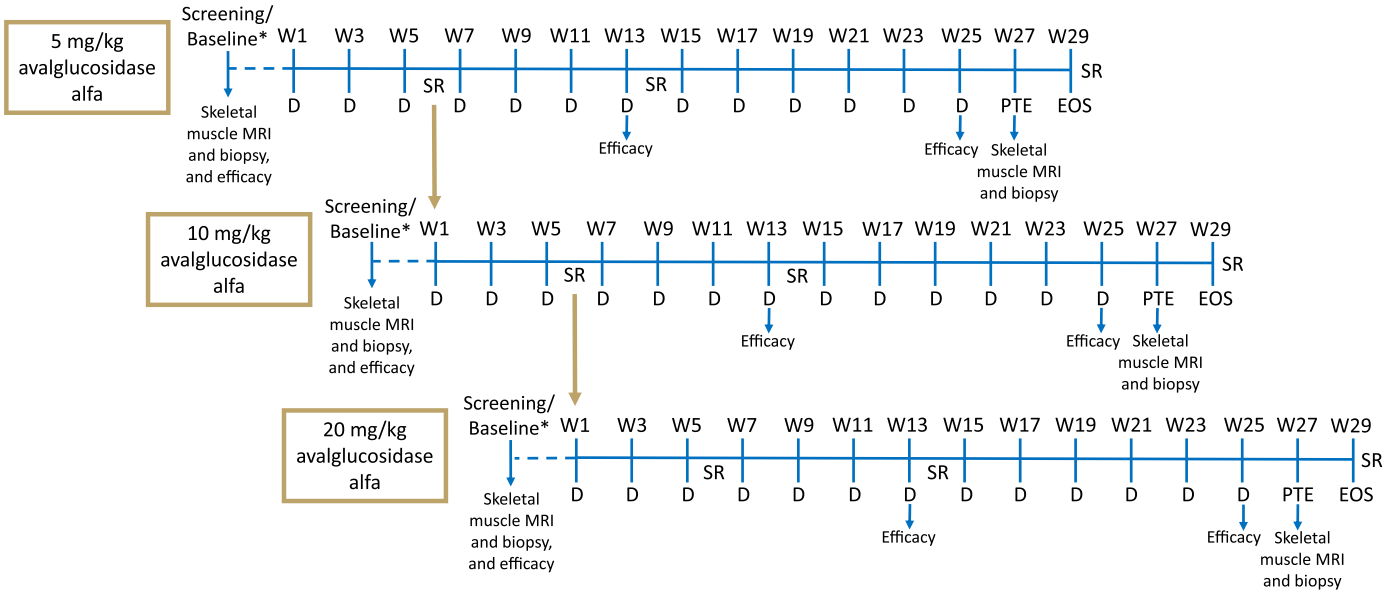

Fig. 2. Study design.
*For the Naïve Group, skeletal muscle MRI and biopsy and all efficacy assessments were performed within 21 days before avalglucosidase alfa initiation; for the Switch Group, these were performed after the last alglucosidase alfa administration and ≤21 days before avalglucosidase alfa treatment.
Exploratory efficacy assessments included: pulmonary function testing; 6-min walk test; Gait, Stair, Gowers' maneuver, Chair test; Gross Motor Function Measure-88; Quick Motor Function Test; hand-held dynamometry test; and Pediatric Quality of life Inventory Multidimensional Fatigue Scale – Adult Report.
W, Week; D, avalglucosidase alfa infusion; PTE, post-treatment evaluation; EOS, end of study visit; SR, safety review by Data Monitoring Committee.

initial rate and gradually increasing if there were no signs of infusion-associated reactions (IARs), up to a maximum of approximately 7 mL/kg/h; total infusion time depended on dose. For management of mild IARs, infusion rate reductions or temporary interruptions were allowed. For moderate-to-severe or recurrent IARs, the investigator could consider using pre-treatment medications (i.e., antihistamines, antipyretics, and/or glucocorticoids), in addition to infusion rate reductions, interruptions, or discontinuation, if necessary.

Study duration for each patient was approximately 41 weeks from screening to study end. This included: screening within 90 days before inclusion and treatment initiation, a baseline evaluation, a 24-week treatment period (Weeks 1–25), a post-treatment evaluation 2 weeks after the last infusion (Week 27), and an end-of-study visit 4 weeks after the final infusion (Week 29).

The study protocol was approved by Independent Ethics Committees/Institutional Review Boards at participating centers (Supplementary Table S1). All patients provided written informed consent.

2.2. Safety

Patients were continuously monitored throughout the study for safety, including IARs, via patient-reported and/or investigator-observed adverse events (AEs). Standard clinical laboratory evaluations (biochemistry, hematology, urinalysis, and vital signs) were assessed at screening/baseline and at each biweekly clinic visit from Week 1 through Week 25. Patients had a physical examination, 12-lead electrocardiogram (ECG), and were weighed at screening/baseline; physical examinations were also made at Weeks 13 and 25, and ECG and body weight measurements at Weeks 1, 13, and 25. Blood samples to test for anti-avalglucosidase alfa antibodies, and for neutralizing antibodies in anti-avalglucosidase alfa antibodies-positive patients, were collected at baseline (within 24 h before the first infusion) and at Weeks 1, 5, 9, 13, 17, 21, 25, and 27. In addition, for Switch Group patients, samples at Weeks 1 and 25 were analyzed for anti-alglucosidase alfa antibodies. IgE, complement, serum tryptase, and circulating immune complexes were tested only in patients with potential hypersensitivity IARs.

2.3. Pharmacokinetics

Blood samples for pharmacokinetic analysis were collected at Weeks 1 (Day 1), 13, and 25, prior to avalglucosidase alfa infusion, immediately before the infusion rate changed from 1 to 3 mg/kg/h, from 3 to 5 mg/kg/h, and from 5 to 7 mg/kg/h, immediately before the end of infusion (0 h), and at 1, 2, 4, 8, 12, 16, 20, 24, 32, 40, and 48 h after the end of infusion. Avalglucosidase alfa activity was determined using a qualified, sensitive fluorometric assay, with 4-methylumbelliferyl-α-D-glucoside pyranoside as the substrate; the lower limit of quantification was 13 ng/mL.

The following pharmacokinetic parameters were calculated for plasma concentrations of avalglucosidase alfa after single and multiple doses using non-compartmental methods: maximum plasma concentration observed (C_{max}); time to reach C_{max} (t_{max}); area under the plasma concentration–time curve (AUC) calculated using the trapezoidal method from time zero to the real-time t_{last} (AUC_{last}); AUC extrapolated to infinity (AUC_{∞}); terminal half-life ($t_{1/2z}$; i.e., the terminal

half-life associated with the terminal slope [λ_z]); apparent total body clearance from plasma (CL); and volume of distribution at steady-state (V_{ss}).

2.4. Pharmacodynamics

The following pharmacodynamic parameters were assessed after single and multiple dose administration of avalglucosidase alfa: skeletal muscle glycogen content by MRI; qualitative (T_1-weighted Mercuri scoring; 1: normal, 2: <30% fat, 3: ≥30% to <60% fat, and 4: ≥60% fat) and quantitative (3-point Dixon, T_2); fasted urinary glucose tetrasaccharide (Hex_4); and exploratory fasted plasma and urine biomarkers.

MRI and skeletal muscle biopsies were performed at baseline (within 21 days before treatment start) and post-treatment (Week 27). Normal-appearing muscle tissue (determined by MRI guidance) was sampled to prevent biopsy of fatty or fibrotic tissue. Glycogen content was measured in skeletal muscle biopsies by computer-assisted histomorphometric analysis (MetaMorph®) of high-resolution light microscopy sections as previously described [20,39]. Briefly, to quantify accurately the distribution of glycogen across the entire specimen, up to 10 epoxy resin blocks were processed per sample-time point for each patient. One slide from each block was analyzed; values obtained were averaged to obtain a mean ± standard deviation (SD) for each patient-time point. Data were expressed as the % tissue area occupied by glycogen.

Fasted urine Hex_4 and exploratory urine biomarker samples were collected at baseline, and every other week during treatment, and the exploratory plasma samples were collected at baseline, and Weeks 1, 3, 5, 9, 13, 17, 21, and 25; all samples were taken within 24 h before avalglucosidase alfa infusion. The exploratory urine and plasma biomarker analyses are pending and will be reported separately in the future.

2.5. Exploratory efficacy

Avalglucosidase alfa efficacy on functional capacity and strength was evaluated using several functional outcome measures. Exploratory assessments were made at baseline and at Weeks 13 and 25 within 24 h before avalglucosidase alfa infusion; changes from baseline to Weeks 13 and 25 were calculated.

Pulmonary function testing followed American Thoracic Society guidelines [40] and included the assessment of FVC, forced expiratory volume in 1 s (FEV_1), maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and peak expiratory flow (PEF) in the upright and supine positions (only upright data are reported). The % of predicted normal values for MIP and MEP were calculated according to Evans and Whitelaw [41] and for the FVC, FEV_1, and PEF according to Hankinson et al. [38].

The 6-min walk test (6MWT) was conducted according to American Thoracic Society guidelines [42]. Changes in meters walked from baseline and changes in % predicted from baseline were calculated at each assessment point. Predicted values were calculated according to Enright and Sherrill [43].

The Gait, Stair, Gowers' Maneuver, Chair (GSGC) test [44] consists of four functional tests (gait, climbing stairs, Gowers' maneuver, and arising from a chair). Each test is scored from 1 (normal function) to 7 (poor function) and the total score calculated by summing the four test scores. Missing tasks were imputed as if subjects could not perform them. Gross Motor Function Measure-88 (GMFM-88) assessment was developed specifically to detect quantitative changes in gross motor function [45]. Of the five dimensions to the GMFM-88, two were evaluated in this study: Dimensions D (standing) and E (walking, running, and jumping; GMFM-88-DE). The Quick Motor Function Test (QMFT) [46], is an observer-administered test comprising 16 items specifically difficult for Pompe disease patients. Items are scored individually on a 5-point ordinal scale (0–4), with a total for all items of 0–64 points; lower scores indicate worse motor function. For the hand-held dynamometry (HHD) test, the examiner held the dynamometer stationary while the patient exerted a maximal force against it, making a gradual increase in force and then completing a 4–5 s isometric hold. Limb tests (shoulder, elbow, hip, knee, and ankle) and grip strength were completed bilaterally because dominant and non-dominant limbs may differ. Each muscle group was measured twice and the highest score analyzed. Individual test scores were summed for the upper and lower body.

The 18-item Pediatric Quality of Life Inventory Multidimensional Fatigue Scale – Adult Report, Standard Version (PedsQL™) [47] encompasses three subscales: General Fatigue, Sleep/Rest Fatigue, and Cognitive Fatigue. Six general fatigue items measure lack of strength and endurance to complete activities of daily living, six sleep/rest fatigue items measure sleep problems and number of naps/day, and six cognitive fatigue items measure the impact of fatigue on attention and memory. Items are evaluated on a 5-point Likert scale, reverse-scored, and linearly transformed to a 1–100 scale. A total score was calculated as the mean derived from the sum of the transformed items divided by the number of items answered on the scales. Higher scores denote better health-related quality of life.

2.6. Statistical methods

In total, 21 patients were planned empirically to complete the study; no formal sample size calculations were performed. The full analysis set (all patients who received ≥1 complete infusion of avalglucosidase alfa), the safety analysis set (all patients who received any amount of avalglucosidase alfa) and the pharmacokinetic analysis set (patients without any major deviations related to study drug administration who had available pharmacokinetic data) comprised the same patients. Demographic and baseline data for medical/surgical history and Pompe disease history were summarized using summary statistics for continuous variables and frequency

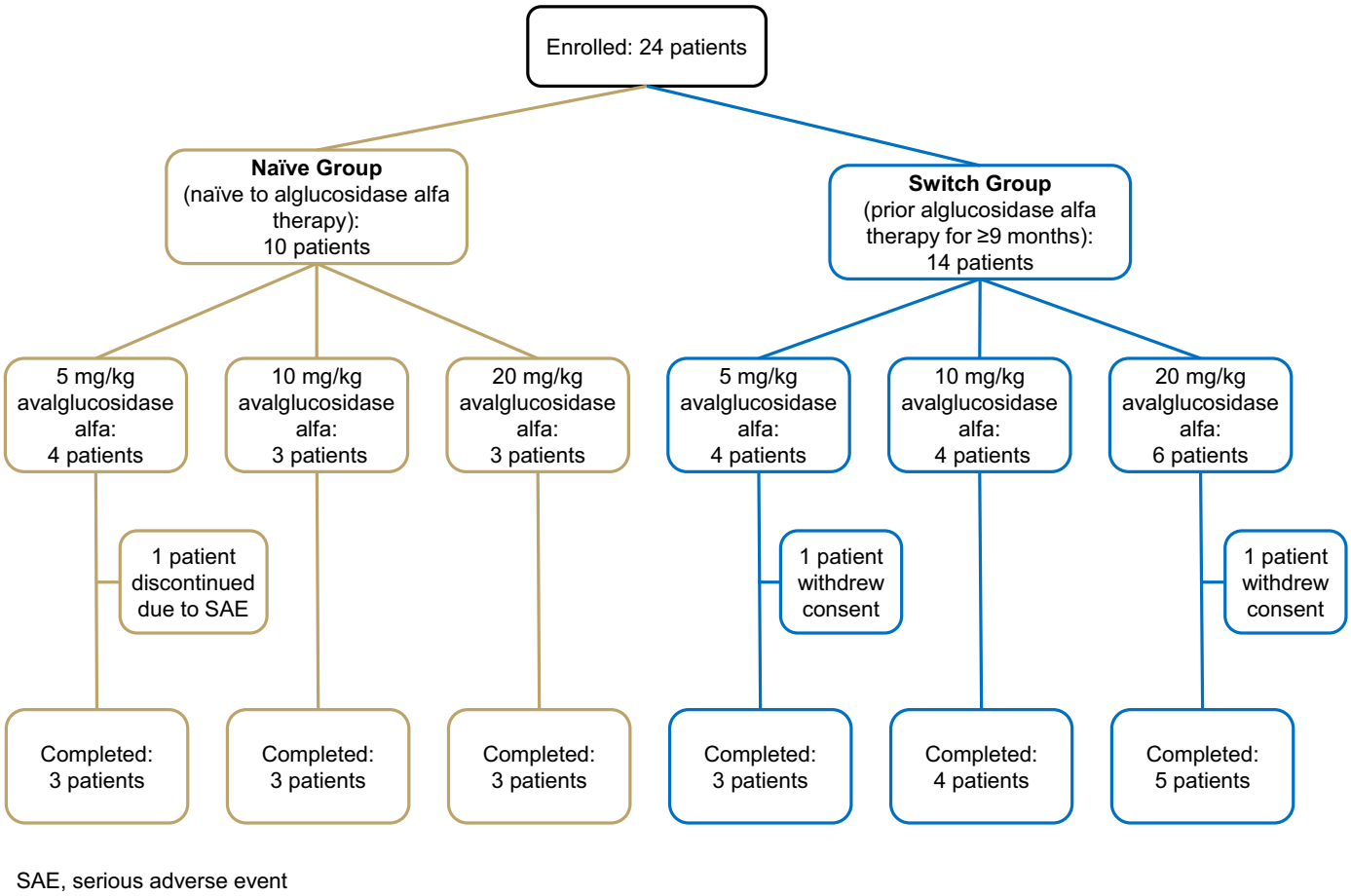

Fig. 3. Flow diagram for disposition of patients during the study.

distribution for categorical variables. Safety evaluation was based on a review of descriptive statistics and individual data for AEs, immunogenicity, clinical laboratory, vital sign, and ECG parameters. Exploratory functional efficacy variables measured at baseline, Week 13, and Week 25 were summarized using summary statistics. Non-parametric methods were used as part of a sensitivity analysis. We found the results robust, but choose to provide the parametric method as it better models the data.

3. Results

3.1. Study sites

The study was carried out at 17 centers (seven in the United States, three in France, three in Germany, and at one center each in Belgium, Denmark, Netherlands, and the United Kingdom). Study sites and NEO1 investigators are listed in Supplementary Table S2.

3.2. Patient disposition

The first patient was enrolled into the study on August 19, 2013 and the last completed the study on February 25, 2015. In total, 24 patients were enrolled and treated (Fig. 3); 10 patients in the Naïve Group (5 mg/kg, n=4; 10 mg/kg, n=3; 20 mg/kg, n=3) and 14 patients in the Switch Group (5 mg/kg, n=4; 10 mg/kg, n=4; 20 mg/kg, n=6). In the Naïve Group, 1 patient in the 5 mg/kg group discontinued treatment due to serious AEs (SAEs) of respiratory distress and chest discomfort; these occurred during the ninth avalglucosidase alfa infusion and were considered IARs. In the Switch Group, 2 patients discontinued treatment for non-AE-related reasons (1 patient in the 5 mg/kg group following the last avalglucosidase alfa infusion and 1 patient in the 20 mg/kg group following their eighth avalglucosidase alfa infusion).

3.3. Demographics and Pompe disease history

Patient demographics and Pompe disease history for the overall Naïve and Switch Groups are presented in Table 1 and by treatment groups in Supplementary Table S3. Overall, in the Naïve and Switch Groups, 30% (3/10) and 64% (9/14), respectively, of patients were male, and most were Caucasian (88% [21/24]). A confirmed Pompe disease family history was recorded in 42% (10/24) of patients and 38% (9/24) had an affected sibling.

Mean±SD age of patients at Pompe disease diagnosis was 43.3±23.8 years for the Naïve Group and 36.3±16.4 years for the Switch Group. Median ages of patients at Pompe disease diagnosis were similar for the two groups (Naïve Group: 36.4

Table 1
Demographic characteristics and Pompe disease history.

Parameter	Naïve Group (alglucosidase alfa treatment-naïve Pompe disease patients; *n*=10)	Switch Group (Pompe disease patients previously treated with alglucosidase alfa for ≥9 months; n=14)
Age at study enrollment, years, mean ± SD	44.8 ± 20.26	46.7 ± 14.11
Sex, female/male, *n* (%)	7 (70)/3 (30)	5 (36)/9 (64)
Race, *n* (%)		
Black or African American	0 (0)	1 (7)
White	8 (80)	13 (93)
Multiple	1 (10)	0 (0)
Other	1 (10)	0 (0)
Hispanic/non-Hispanic, n (%)	0 (0)/10 (100)	0 (0)/14 (100)
Height, cm, mean ± SD	170.8 ± 8.20	176.3 ± 10.82
Weight, kg, mean ± SD	65.0 ± 9.85	76.7 ± 15.84
Body mass index, kg/m^2, mean ± SD (median [range])	22.3 ± 3.14 (23.2 [17.0−26.1])	24.6 ± 3.69 (24.1 [17.0−31.0])
Age at Pompe disease diagnosis, years, mean ± SD (median [range])	43.3 ± 23.79* (36.4 [15.8, 78.2])	36.3 ± 16.39† (34.2 [3.4, 62.9])
Pompe disease family history, yes/no, n (%)	4 (40)/6 (60)	6 (43)/8 (57)
If yes, relationship to patient, siblings/cousins, *n* (%)	3 (30)/1 (10)	6 (43)/0 (0)
Assistive walking devices and orthoses, *n* (%)		
None	8 (80)	11 (79)
Rolling walker	1 (10)	1 (7)
Straight cane	0 (0)	2 (14)
Other: two walking sticks (poles)	1 (10)	0 (0)

SD, standard deviation.
* *n*=8.
† *n*=9.

[range 15.8, 78.2] years; Switch Group: 34.2 [range 3.4, 62.9] years). Patients entered the current study at various times after initial diagnosis (mean age at study enrollment was 44.8±20.3 years for the Naïve Group and 46.7±14.1 years for the Switch Group). In the Switch Group, 9 patients had full data for pre-study alglucosidase alfa treatment duration, which ranged from 0.9 to 7.9 years. Overall, 21% (5/24) of patients used a walking device at baseline (Naïve Group: 20%; Switch Group: 21%).

During the study, 7 patient samples were genotyped for pathogenic *GAA* variants and the remaining genotypes were retrieved from historic patient data. All genotypes were consistent with an LOPD diagnosis. Eighteen patients had the common c.-32-13T>G splice variant in compound heterozygosity with another *GAA* pathogenic variant. Six patients had two pathogenic *GAA* variants not including c.-32-13T>G.

3.4. Safety

3.4.1. Adverse events

In both groups, avalglucosidase alfa was generally well-tolerated at all doses; no deaths or life-threatening SAEs were reported. Most treatment-emergent AEs (TEAEs) were non-serious, with mild-to-moderate intensity (95% [169/176]). Regardless of relationship to treatment, overall, 80% (8/10) of Naïve Group patients and 86% (12/14) of Switch Group patients experienced ≥1 TEAE, with respective group totals of 83 and 93 TEAEs. TEAEs considered related to the study drug (Table 2); occurred in 60% (6/10) of Naïve Group patients (16 events), and in 50% (7/14) of Switch Group patients (26 events). The number of patients experiencing ≥1 TEAE and the number of events during the study were similar across all doses and for each group.

In the Naïve Group, across all doses, common TEAEs regardless of their relationship to study drug included headache (8 events in 4 patients), rash (8 events in 3 patients), dizziness (7 events in 3 patients), nausea (4 events in 3 patients), oral herpes (4 events in 1 patient), dysmenorrhea (4 events in 2 patients), and diarrhea (3 events in 3 patients). In the Switch Group, across all doses, common TEAEs regardless of their relationship to study drug included headache (10 events in 3 patients), myalgia (6 events in 1 patient), musculoskeletal pain (4 events in 3 patients), falls (4 events in 2 patients), and nasopharyngitis (3 events in 3 patients).

In the Naïve Group, there were two study drug-related SAEs (respiratory distress and chest discomfort) reported by 1 patient in the 5 mg/kg group at the ninth infusion (Week 17); both were study drug-related IARs and led to treatment discontinuation and study withdrawal. The TEAE of chest discomfort was considered to be severe and began 3 min into infusion along with a cough (moderate). Three minutes later the TEAE of respiratory distress began and flushing 1 min later; both were considered moderate in intensity. All TEAEs resolved, with the flushing and chest discomfort resolving approximately 7–9h after onset, the TEAE of cough resolving approximately 1.5 days later, and respiratory distress resolving 3 days later. In addition, the patient reported dizziness and nausea, which occurred following

Table 2
Number (%) of patients with study drug-related treatment-emergent adverse events by Medical Dictionary for Regulatory Activities (MedDRA) preferred term.

MedDRA: Preferred term	Naïve Group (alglucosidase alfa treatment-naïve Pompe disease patients; *n*=10)	Switch Group (Pompe disease patients previously treated with alglucosidase alfa for ≥9 months; n=14)
	Patients, *n* (%)	Patients, *n* (%)
Any events	6 (60)	7 (50)
Fatigue	2 (20)	1 (7)
Nausea	2 (20)	0 (0)
Abdominal pain	0 (0)	1 (7)
Asthenia	0 (0)	1 (7)
Balanoposthitis	0 (0)	1 (7)
Chest discomfort	1 (10)	0 (0)
Cough	1 (10)	0 (0)
Diarrhea	0 (0)	1 (7)
Dizziness	1 (10)	1 (7)
Dyspnea	1 (10)	1 (7)
Erythema	1 (10)	0 (0)
Facial pain	0 (0)	1 (7)
Flushing	1 (10)	0 (0)
Gastroesophageal reflux disease	1 (10)	0 (0)
Headache	1 (10)	1 (7)
Hypersensitivity	0 (0)	1 (7)
Hypotension	0 (0)	1 (7)
Infusion site reaction	0 (0)	1 (7)
Muscle spasms	0 (0)	1 (7)
Myalgia	1 (10)	1 (7)
Pruritus	0 (0)	1 (7)
Pulmonary function test decreased	0 (0)	1 (7)
Rash	1 (10)	1 (7)
Respiratory distress	1 (10)	0 (0)
Somnolence	0 (0)	1 (7)

treatment with 50 mg IV diphenhydramine hydrochloride. The patient received epinephrine during the IAR and had not received pre-infusion antipyretics, antihistamines, or steroids.

In the Switch Group, 1 patient in the 5 mg/kg group experienced one treatment-emergent SAE (gastrointestinal hemorrhage), which did not lead to treatment discontinuation or study withdrawal, and was not considered study drug-related.

For both groups, the majority of TEAEs were mild across all dose levels. Fifteen of the 24 enrolled patients reported having used concomitant medications during the study for TEAEs.

3.4.2. Infusion-associated reactions

Overall, 25 IARs were reported/identified in 8 patients across both groups.

In the Naïve Group, 40% (4/10) of patients reported a total of 11 IARs; the most commonly reported (2/11 IARs) was flushing; all other IARs occurred once. All IARs were non-serious except for the two IARs in the patient at 5 mg/kg that led to study withdrawal as described in Section 3.4.1. This patient experienced six IARs (chest discomfort [SAE], cough, respiratory distress [SAE], flushing, dizziness, and nausea) at the ninth avalglucosidase alfa infusion (Week 17). The patient had also experienced IARs of rash following the second infusion and flushing following the eighth infusion; at the time both were considered possibly study drug-related. This patient was negative for anti-avalglucosidase alfa antibodies at screening and seroconverted at Week 13 with a titer of 1600; immediately before the ninth infusion the titer was 3200. Further samples collected following the ninth infusion were negative for anti-avalglucosidase alfa IgE antibodies, positive for complement activation, and tryptase (3.6 μg/L) was within the normal range (i.e., ≤12.5 μg/L). None of the anti-avalglucosidase alfa antibodies inhibited enzyme uptake or enzymatic activity.

One Naïve Group patient at 20 mg/kg experienced an IAR of erythema following the fourth infusion. The patient was negative for anti-avalglucosidase alfa antibodies at screening and seroconverted at Week 9, with sustained antibody titers ranging from 400 to 800 until study end. Samples collected following this event were negative for anti-avalglucosidase alfa IgE antibodies and complement activation, and tryptase (2.4 μg/L) was within the normal range. None of the anti-avalglucosidase alfa antibodies inhibited enzyme uptake or enzymatic activity.

In the Switch Group, 29% (4/14) of patients reported 14 IARs; all were non-serious. The most common IARs were myalgia (6 events in 1 patient) and headache (2 events in 1 patient), with all other IARs occurring only once. Three IARs (hypersensitivity, pruritus, and generalized rash) were reported by 1 Switch Group patient at 20 mg/kg during the thirteenth avalglucosidase alfa infusion. Samples collected

in association with these IARs were negative for anti-avalglucosidase alfa IgE antibodies, positive for complement activation, and tryptase (4.7 μg/L) was within the normal range.

3.4.3. ECG assessments and clinically relevant laboratory abnormalities

There were no individual clinically relevant abnormalities in ECG data for any patients. In the Switch Group, 2 patients had clinically relevant laboratory abnormalities. One patient (10 mg/kg group) had hypokalemia in Week 15, which was considered a mild TEAE, unlikely related to avalglucosidase alfa, and was resolved by Week 17, with no requirement for concomitant medication. The other patient (5 mg/kg group) had severe TEAEs of hypovolemia and anemia, which began on Day 48, 3 days following the fourth infusion; this is the same patient who experienced a SAE of a gastrointestinal bleed on Day 48 (as mentioned in Section 3.4.1.) Neither the gastrointestinal bleed nor the associated TEAEs were considered related to avalglucosidase alfa. The hypovolemia and anemia resolved after approximately 3 and 5 months, respectively; both were treated with concomitant medications.

3.4.4. Immunogenicity

In the Naïve Group, 90% (9/10) of patients developed antibodies to avalglucosidase alfa during treatment, with a median peak titer of 1600 (range 200–25,600) observed across all treatments. Mean time to seroconversion from first infusion was 56.9±14.9 (median 56 [range 28–87]) days, with the peak titer occurring on average 108.4±37.7 (median 89 [range 77–175]) days from first infusion. After seroconversion, 1 patient had sustained and elevated titers (defined by protocol as a peak titer ≥25,600, and a last titer that was equal to, higher than, or one 2-fold dilution level lower than the peak titer); this patient's peak titer was 25,600. This patient showed improvement on pulmonary and motor function testing. Two patients who had seroconverted had decreased titers later (i.e., at least 4-fold lower than the peak titer); 1 of these patients decreased by 8-fold (three dilution levels), and the other patient was negative for antibodies at the final time point after a peak titer of 1600, suggesting that this patient may have tolerized. One patient (5 mg/kg) tested positive for uptake-inhibitory antibodies at Week 27; no anti-avalglucosidase alfa antibodies inhibited enzymatic activity. As noted in Section 3.4.2., 2 Naïve Group patients tested negative for anti-avalglucosidase alfa IgE.

In the Switch Group, 36% (5/14) of patients tested positive for antibodies to avalglucosidase alfa at screening, with a maximum titer of 1600 observed at Week 1. Titers in 2 of the 5 patients demonstrated a treatment-boosted response with 4- and 8-fold increased titers from the baseline levels, with a maximum titer of 12,800. Of the 9 patients who tested negative at screening and Week 1 for anti-avalglucosidase alfa antibodies, 2 (22%) patients seroconverted after Week 1, with a maximum titer of 1600. Overall mean time to avalglucosidase alfa seroconversion was 4.7±46.6 (median –12.0 [range –44.0 to 84.0]) days from first on-study infusion (Week 1). Peak titers for patients who seroconverted or exhibited a boosted response (n=4) occurred on average 143±27.0 (median 142 [range 111–175]) days after first infusion. For the 5 patients with pre-study positive antibodies and 2 patients who seroconverted during the study, the median peak titer was 400 (range 200–12,800). No patient had sustained and elevated titers, and 2 patients (14.3%) had decreased titers (which may be within-assay variability, as none decreased >2-fold). None of the anti-avalglucosidase alfa antibodies inhibited enzyme uptake or enzymatic activity. At Week 1, 50% (7/14) of Switch Group patients tested positive for IgG antibodies to alglucosidase alfa, with a maximum titer of 6400. Of the 7 Switch Group patients who tested negative at Week 1 for anti-alglucosidase alfa antibodies, 3 patients seroconverted with antibodies to alglucosidase alfa during avalglucosidase alfa treatment, with a maximum observed titer of 3200 (range 1600–3200). As noted in Section 3.4.2., 1 patient in the Switch Group tested negative for anti-avalglucosidase alfa IgE.

3.5. Pharmacokinetics

Pharmacokinetics for the Naïve and Switch Groups are presented in Tables 3 and 4, respectively. In both the Naïve and Switch Groups, avalglucosidase alfa exposure increased with dose (in the 20 mg/kg group the AUC was approximately 5–6 × greater than in the 5 mg/kg); mean systemic CL ranged from 0.92 to 1.28 L/h and 1.0 to 1.57 L/h, respectively, and mean V_{ss} ranged from 2.40 to 3.02 L and 2.83 to 3.71 L, respectively. Within each treatment group, the pharmacokinetic parameters appeared similar at Weeks 1, 13, and 25, indicating no apparent effect of every-other-week dosing on avalglucosidase alfa pharmacokinetics. The pharmacokinetic parameters also appeared generally similar between the Naïve and Switch groups.

The mean avalglucosidase alfa plasma concentration–time profiles are presented in Fig. 4A–C. In both groups, avalglucosidase alfa plasma concentrations appeared to decline monoexponentially from 8 to 12 h after the end of the infusion; mean $t_{½z}$ ~1.0 h for each group (range 0.66–1.53 h). However, consistent avalglucosidase alfa plasma concentrations of approximately 20 ng/mL were observed from approximately 12 h post infusion to the last sample taken, likely representing the endogenous enzyme levels. For both groups, the accumulation ratios at Weeks 13 and 25, based on AUC values, were generally close to 1.0, which was consistent with the short $t_{½z}$ and every-other-week dosing regimen.

3.6. Pharmacodynamics

Quadriceps biopsies were available at baseline and Week 27 for 9 patients who completed the study in each of the Naïve and Switch groups. Mean±SD quadriceps muscle

Table 3
Avalglucosidase alfa pharmacokinetic parameters for alglucosidase alfa treatment-naïve Pompe disease patients (Naïve Group).

Parameter	Week*	Avalglucosidase alfa		
		5 mg/kg (n=4, Week 1; n=3 Weeks 13 and 25)	10 mg/kg (n=3)	20 mg/kg (n=3)
C_{max}, ng/mL, mean ± SD (geometric mean) [CV%]	1	82,300 ± 6690 (82,100) [8.1]	190,000 ± 40,100 (187,000) [21.1]	302,000 ± 107,000 (291,000) [35.6]
	13	98,700 ± 38,900 (93,700) [39.4]	151,000 ± 29,700 (149,000) [19.7]	357,000 ± 185,000 (329,000) [51.7]
	25	89,100 ± 11,000 (88,600) [12.3]	162,000 ± 26,500 (161,000) [16.4]	350,000 ± 105,000 (341,000) [29.9]
t_{max}, h, median (min−max)	1	1.71 (1.47−2.58)	2.30 (2.23−2.30)	3.83 (3.75−4.00)
	13	1.60 (1.50−1.80)	2.33 (1.48−2.35)	3.92 (3.75−4.00)
	25	1.43 (1.43−1.62)	2.35 (2.35−2.45)	3.92 (3.75−4.50)
AUC_{last}, ng·h/mL, mean ± SD (geometric mean) [CV%]	1	259,000 ± 37,600 (259,000) [14.5]	529,000 ± 79,600 (525,000) [15.0]	1,520,000 ± 806,000 (1,400,000) [53.0]
	13	285,000 ± 82,900 (276,000) [29.1]	529,000 ± 41,800 (528,000) [7.9]	1,660,000 ± 1,030,000 (1,470,000) [62.2]
	25	264,000 ± 50,200 (261,000) [19.0]	565,000 ± 89,800 (560,000) [15.9]	1,560,000 ± 637,000 (1,490,000) [40.7]
AUC, ng·h/mL, mean ± SD (geometric mean) [CV%]	1	259,000 ± 37,600 (257,000) [14.5]	529,000 ± 79,600 (525,000) [15.0]	1,520,000 ± 806,000 (1,400,000) [53.0]
	13	285,000 ± 82,900 (276,000) [29.1]	529,000 ± 41,900 (528,000) [7.9]	1,660,000 ± 1,030,000 (1,470,000) [62.2]
	25	264,000 ± 50,200 (261,000) [19.0]	565,000 ± 89,800 (560,000) [15.9]	1,560,000 ± 637,000 (1,490,000) [40.7]
$t_{½z}$, h, mean ± SD (geometric mean) [CV%]	1	0.784 ± 0.369 (0.727) [47.1]	0.833 ± 0.493 (0.750) [59.2]	0.778 ± 0.217 (0.757) [27.9]
	13	1.34 ± 1.05 (1.10) [78.2]	0.738 ± 0.133 (0.730) [18.1]	1.34 ± 0.753 (1.20) [56.0]
	25	0.777 ± 0.0455 (0.776) [5.9]	0.856 ± 0.235 (0.835) [27.5]	1.03 ± 0.242 (1.01) [23.5]
CL, mL/h, mean ± SD (geometric mean) [CV%]	1	1260 ± 203 (1240) [16.2]	1240 ± 386 (1190) [31.3]	989 ± 278 (959) [28.1]
	13	1220 ± 307 (1190) [25.2]	1280 ± 261 (1270) [20.3]	954 ± 335 (909) [35.1]
	25	1230 ± 229 (1220) [18.5]	1180 ± 176 (1170) [14.9]	917 ± 214 (901) [23.4]
V_{ss}, mL, mean ± SD (geometric mean) [CV%]	1	2620 ± 396 (2600) [15.1]	2480 ± 626 (2420) [25.3]	2900 ± 439 (2870) [15.2]
	13	2650 ± 803 (2540) [30.3]	3020 ± 766 (2960) [25.4]	2910 ± 661 (2860) [22.7]
	25	2400 ± 316 (2390) [13.1]	2670 ± 109 (2670) [4.1]	2930 ± 233 (2930) [7.9]
t_{last}, h, median (min−max)	1	9.63 (9.57−17.77)	12.47 (12.43−12.47)	19.75 (13.92−20.00)
	13	14.69 (9.83−33.50)	14.33 (12.67−15.28)	19.75 (15.25−23.92)
	25	12.53 (9.62−13.82)	14.10 (12.60−18.47)	20.50 (15.92−23.75)
C_{last}, ng/mL, mean ± SD (geometric mean) [CV%]	1	65.8 ± 102 (29.2) [154.4]	13.0 ± 0.00 (13.0) [0.0]	23.7 ± 8.08 (22.8) [34.2]
	13	18.5 ± 7.14 (17.6) [38.6]	19.3 ± 3.21 (19.2) [16.6]	36.0 ± 4.58 (35.8) [12.7]
	25	64.3 ± 72.5 (42.1) [112.7]	24.3 ± 7.77 (23.6) [31.9]	45.3 ± 15.0 (43.4) [33.1]
Accumulation ratio (AUC)	13	1.10	1.00	1.09
	25	1.02	1.07	1.03

AUC, area under the plasma concentration–time curve extrapolated to infinity; AUC_{last}, area under the plasma concentration–time curve calculated using the trapezoidal method from last time point attributable to the administered drug; CL, apparent total body clearance from the plasma; C_{last}, last concentration above the limit of quantitation considered to be due to administered drug effect; C_{max}, maximum plasma concentration observed; CV, coefficient of variation; SD, standard deviation; $t_{½z}$, terminal half-life; t_{last}, time corresponding to the last time point attributable to the administered drug and above the limit of quantitation, C_{last}; t_{max}, time to reach C_{max}; V_{ss}, mean steady-state volume of distribution.

* Week 1, 1st infusion; Week 13, 7th infusion; Week 25, 13th infusion.

Table 4
Avalglucosidase alfa pharmacokinetic parameters for Pompe disease patients previously treated with alglucosidase alfa for ≥9 months (Switch Group).

Parameter	Week*	Avalglucosidase alfa		
		5 mg/kg (n=4)	10 mg/kg (n=4)	20 mg/kg (n=6, Week 1 and 13; n=5, Week 25)
C_{max}, ng/mL, mean ± SD (geometric mean) [CV%]	1	77,400 ± 22,400 (74,800) [29.0]	168,000 ± 36,800 (165,000) [21.9]	321,000 ± 125,000 (303,000) [38.9]
	13	103,000 ± 42,800 (96,300) [41.4]	171,000 ± 45,100 (166,000) [26.4]	327,000 ± 90,200 (317,000) [27.6]
	25	97,100 ± 36,400 (90,900) [37.6]	164,000 ± 19,100 (163,000) [11.6]	299,000 ± 47,500 (296,000) [15.9]
t_{max}, h, median (min−max)	1	1.84 (1.38−2.60)	2.27 (1.75−2.43)	3.83 (3.68−4.73)
	13	1.64 (1.52−2.58)	2.44 (2.28−2.72)	3.86 (3.58−4.23)
	25	1.97 (1.50−2.62)	2.51 (2.25−3.35)	3.83 (3.68−5.58)
AUC_{last}, ng·h/mL, mean ± SD (geometric mean) [CV%]	1	246,000 ± 81,500 (236,000) [33.1]	631,000 ± 118,000 (622,000) [18.7]	1,500,000 ± 502,000 (1,430,000) [33.4]
	13	296,000 ± 84,200 (288,000) [28.4]	668,000 ± 186,000 (646,000) [27.9]	143,0000 ± 529,000 (1,350,000) [37.0]
	25	306,000 ± 79,900 (298,000) [26.1]	642,000 ± 46,900 (641,000) [7.3]	1,530,000 ± 434,000 (1,480,000) [28.5]
AUC, ng·h/mL, mean ± SD (geometric mean) [CV%]	1	246,000 ± 81,500 (236,000) [33.1]	631,000 ± 118,000 (622,000) [18.7]	1,500,000 ± 502,000 (1,430,000) [33.4]
	13	296,000 ± 84,200 (288,000) [28.4]	668,000 ± 186,000 (646,000) [27.9]	1,430,000 ± 529,000 (1,350,000) [37.0]
	25	306,000 ± 79,900 (298,000) [26.1]	642,000 ± 46,900 (641,000) [7.3]	1,530,000 ± 434,000 (1,480,000) [28.5]
$t_{½z}$, h, mean ± SD (geometric mean) [CV%]	1	0.668 ± 0.299 (0.628) [44.8]	1.03 ± 0.628 (0.920) [61.1]	0.876 ± 0.232 (0.852) [26.5]
	13	0.656 ± 0.253 (0.626) [38.5]	0.838 ± 0.214 (0.821) [25.5]	0.849 ± 0.254 (0.816) [29.9]
	25	1.53 ± 0.520 (1.47) [33.8]	0.712 ± 0.103 (0.706) [14.5]	1.06 ± 0.435 (1.00) [40.9]
CL, mL/h, mean ± SD (geometric mean) [CV%]	1	1570 ± 362 (1530) [23.1]	1280 ± 246 (1270) [19.2]	1060 ± 198 (1050) [18.6]
	13	1290 ± 319 (1260) [24.7]	1280 ± 448 (1230) [35.1]	1160 ± 321 (1120) [27.6]
	25	1240 ± 342 (1210) [27.7]	1230 ± 56.3 (1230) [4.6]	998 ± 204 (982) [20.5]
V_{ss}, mL, mean ± SD (geometric mean) [CV%]	1	3710 ± 1520 (3490) [40.9]	3210 ± 839 (3140) [26.1]	3310 ± 731 (3250) [22.1]
		2830 ± 762 (2750) [26.9]	3280 ± 1080 (3170) [32.9]	3510 ± 952 (3390) [27.2]
	25	2880 ± 704 (2820) [24.4]	3060 ± 114 (3060) [3.7]	3290 ± 755 (3210) [23.0]
t_{last}, h, median (min−max)	1	9.68 (9.60−17.67)	14.47 (14.33−26.50)	17.87 (15.75−21.03)
	13	9.62 (9.60−18.67)	14.48 (14.28−18.67)	17.95 (11.75−21.83)
	25	20.04 (13.58−25.75)	14.34 (12.70−14.35)	16.77 (15.67−21.58)
C_{last}, ng/mL, mean ± SD (geometric mean) [CV%]	1	19.0 ± 5.48 (18.5) [28.8]	15.8 ± 1.71 (15.7) [10.8]	29.7 ± 19.5 (25.4) [65.7]
	13	19.0 ± 4.08 (18.7) [21.5]	19.3 ± 4.57 (18.8) [23.8]	34.8 ± 16.8 (32.0) [48.2]
	25	23.8 ± 12.7 (21.1) [53.6]	22.5 ± 10.5 (21.0) [46.5]	47.8 ± 33.7 (40.3) [70.4]
Accumulation ratio (AUC)	13	1.20	1.06	0.95
	25	1.24	1.02	1.02

AUC, area under the plasma concentration–time curve extrapolated to infinity, AUC_{last}, area under the plasma concentration–time curve calculated using the trapezoidal method from last time point attributable to the administered drug; CL, apparent total body clearance from the plasma; C_{last}, last concentration above the limit of quantitation considered to be due to administered drug effect; C_{max}, maximum plasma concentration observed; CV, coefficient of variation; SD, standard deviation; $t_{½z}$, terminal half-life; t_{last}, time corresponding to the last time point attributable to the administered drug and above the limit of quantitation, C_{last}; t_{max}, time to reach C_{max}; V_{ss}, mean steady-state volume of distribution.

* Week 1, 1st infusion; Week 13, 7th infusion; Week 25, 13th infusion.

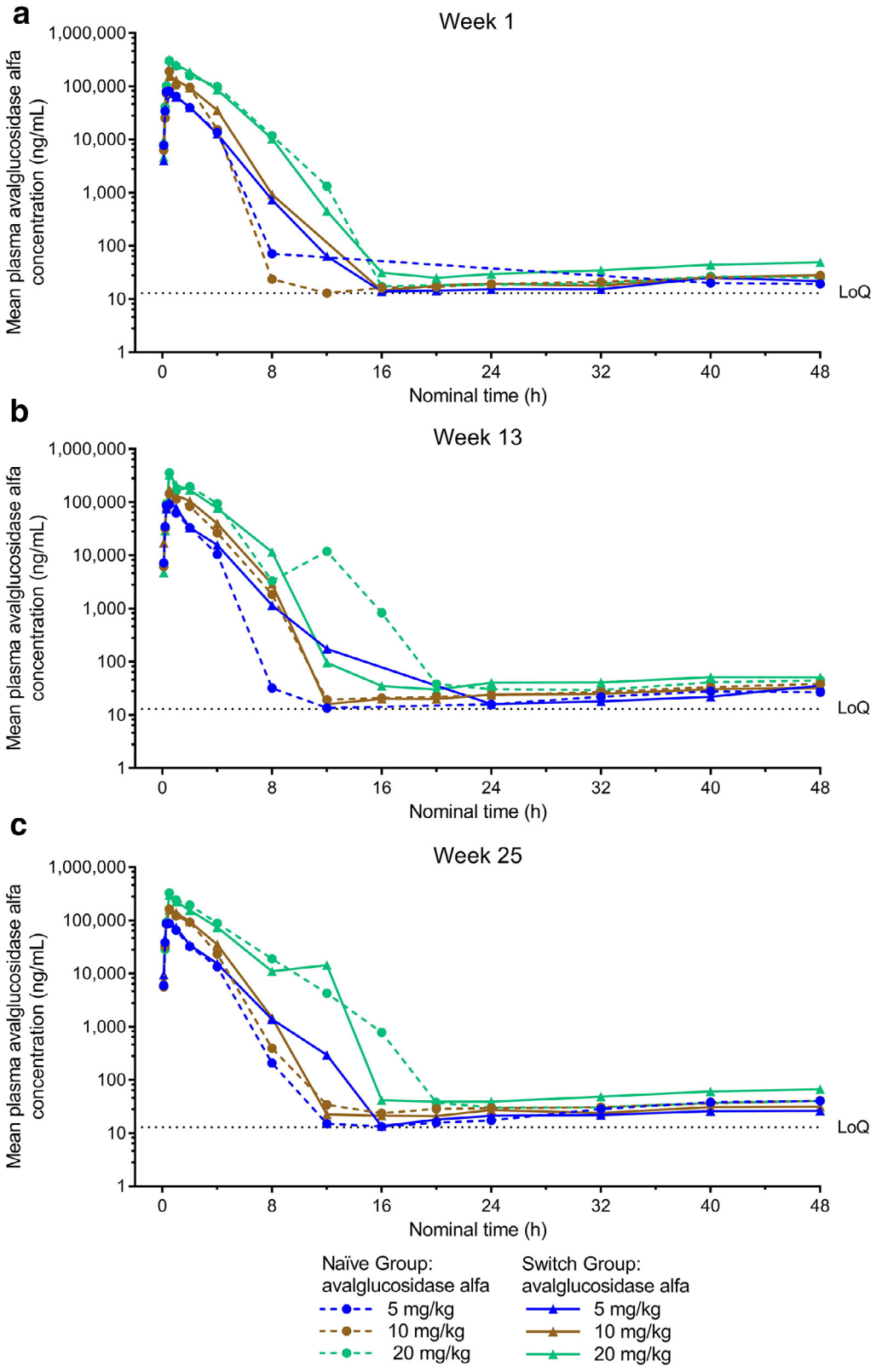

Fig. 4. Mean avalglucosidase alfa plasma concentrations following every-other-week infusions of avalglucosidase alfa (n=3 to 6 per dose level). (a) Week 1, (b) Week 13, and (c) Week 25. Limit of quantitation (LoQ)=13 ng/mL.

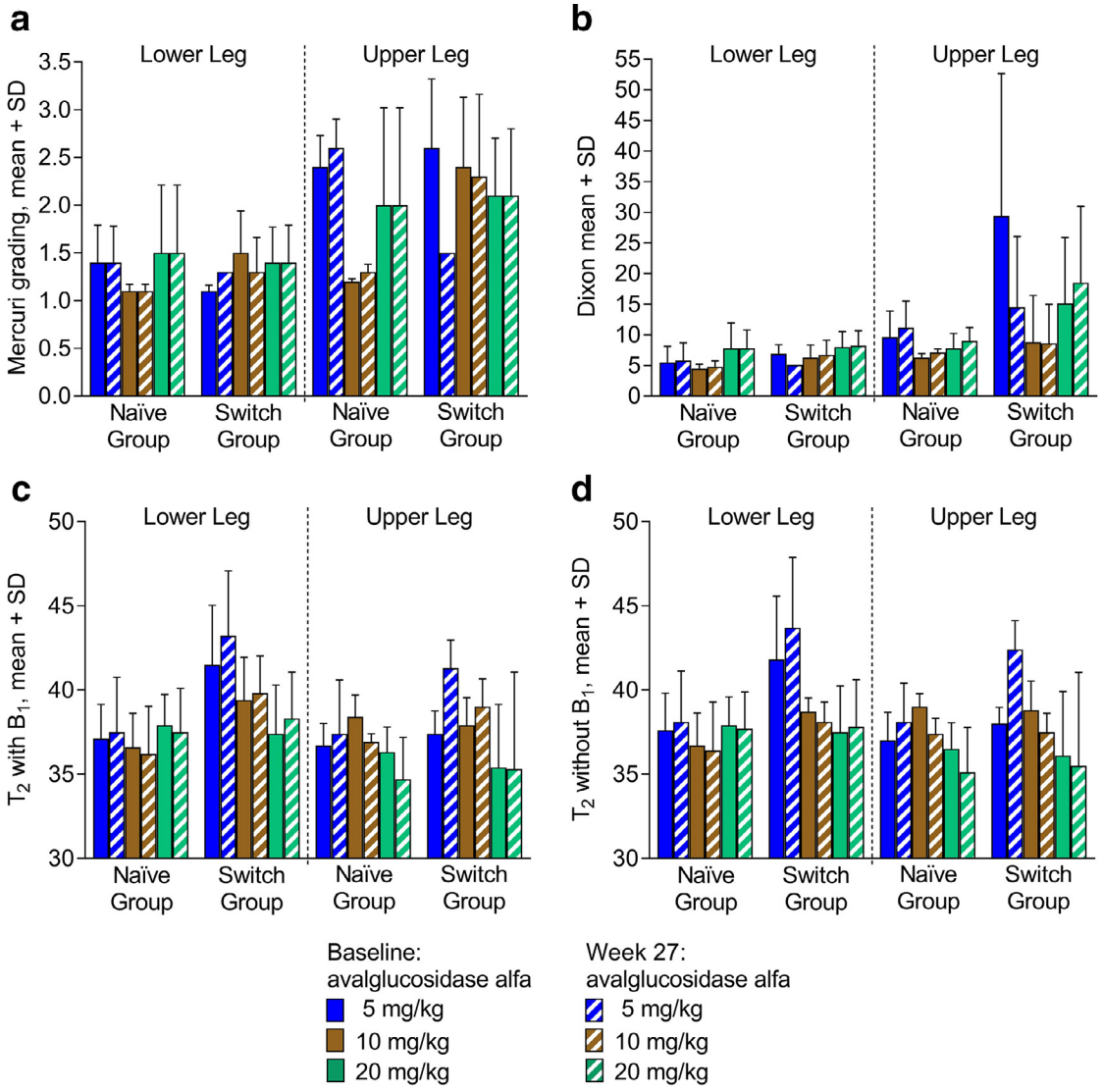

Fig. 5. Evaluation of intact muscle and fatty replacement by MRI (a) Mercuri score, (b) 3-point Dixon, (c) T_2 with B_1, and (d) T_2 without B_1.

glycogen content at baseline was 6.0±7.3% in the Naïve Group and 6.5±7.9% in the Switch Group. For most patients, quadriceps muscle glycogen levels remained unchanged from baseline to Week 27 (see Supplementary Fig. S1A and B).

Urinary Hex_4 concentrations over time for individual patients in the Naïve and Switch groups are shown in Supplementary Fig. S2A and B, respectively. At Week 13, across both groups, mean±SD % changes from baseline in urinary Hex_4 concentrations decreased at all avalglucosidase alfa dose levels, except in the 5 mg/kg Switch Group (4.3±8.8%). At Week 25, the % changes from baseline were: Naïve Group: –30.3±18.6%, –36.0±6.9%, and –13.2±40.6% for the 5, 10, and 20 mg/kg avalglucosidase alfa groups, respectively, and Switch Group: –7.5±38.8%, –12.0±29.7%, and –20.5±27.8% for the 5, 10, and 20 mg/kg avalglucosidase alfa groups, respectively.

Evaluation of intact muscle and fatty replacement in the upper leg (thigh) and lower leg with Mercuri scores, 3-point Dixon, and T_2 MRI with and without B_1 mapping, demonstrated minimal changes throughout the study for both Naïve and Switch Group patients (Fig. 5A–D). Similar findings were observed across both groups. In the Naïve group, some changes from baseline in the upper leg 3-point Dixon parameter at Week 27 corresponded to nominal *p*-values of <0.05 for the 5 and 10 mg/kg, and overall groups (19.4% change [95% CI: 1.0, 37.9], p=0.0438; 20.6% change [95% CI: 14.4, 26.9], p=0.0153; and 15.9% change [95% CI: 6.6, 25.2], p=0.0050, respectively). In the Switch Group at Week 27, some changes from baseline corresponded to nominal p-values of <0.05; these were the for the upper and lower legs T_2 without B_1 for the 5 mg/kg group (12.3% [95% CI: 5.0, 19.6], p=0.0185 and 5.0% [95% CI: 0.5, 9.5], p=0.0413, respectively) and for the lower leg T_2 with B_1 for the overall group (2.5% [95% CI 0.1, 4.8], p=0.0451). For both the Naïve and Switch Groups, all other % changes from baseline at Week 27 for the four pharmacodynamics response parameters in any treatment group or overall did not reach nominal significance.

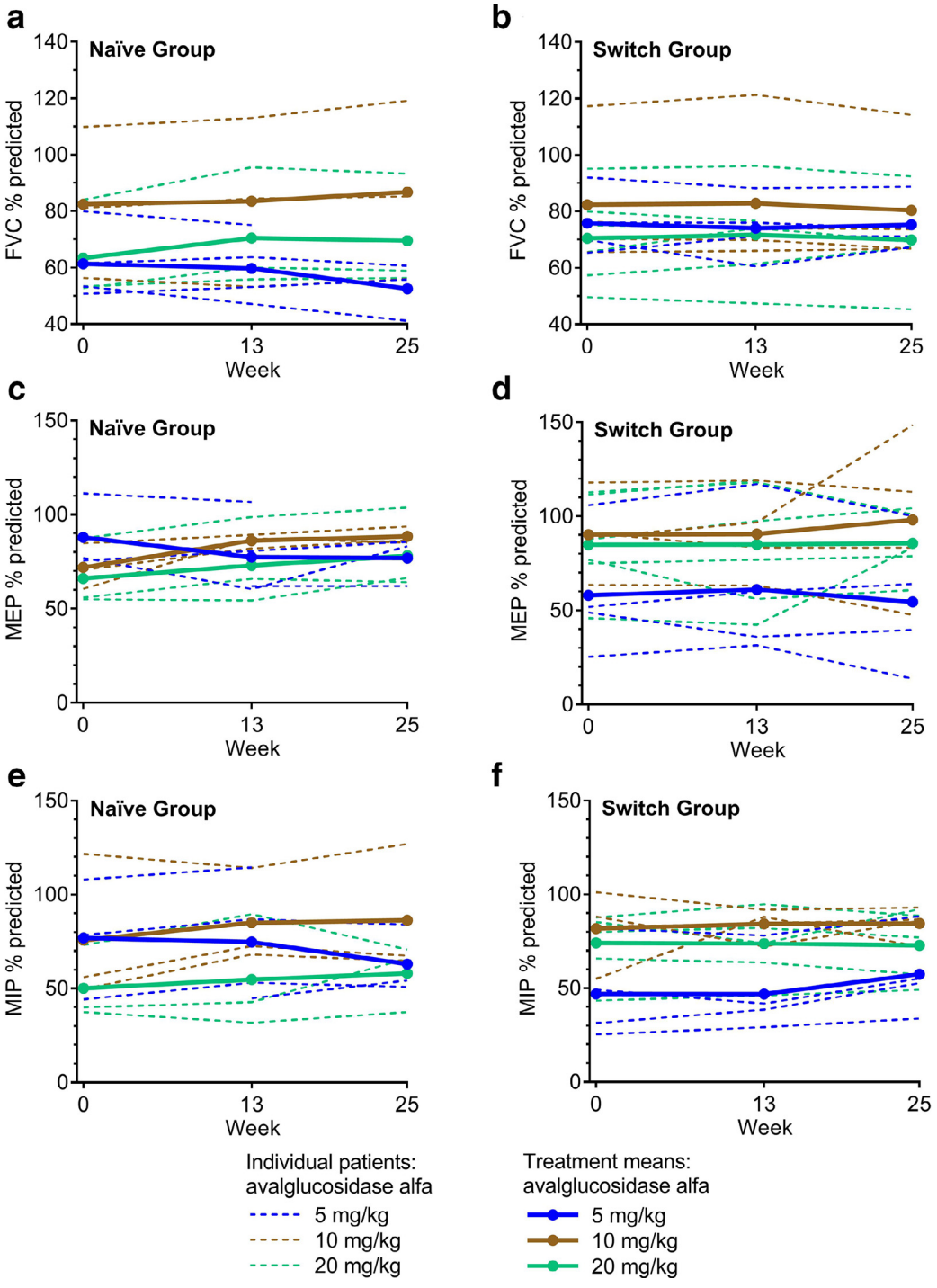

Fig. 6. Upright % predicted forced vital capacity (FVC), maximal expiratory pressure (MEP), and minimal inspiratory pressure (MIP) for individual patients and treatment means.

3.7. Exploratory efficacy

3.7.1. Pulmonary function

Upright % predicted FVC, MEP, and MIP for individual patients along with treatment means at baseline, and Weeks 13 and 25 for the Naïve and Switch Groups are shown in Fig. 6 (see Supplementary Table S4 for the mean±SD, median, minimum, and maximum FVC, MEP, and MIP values at each time point, along with mean % change from baseline at Week 25 for each treatment). In both groups, pulmonary function generally improved or was stable at Week 25 relative to baseline.

3.7.2. 6MWT

The % predicted 6MWT distances for individual patients and treatment means are shown in Fig. 7 (Supplementary

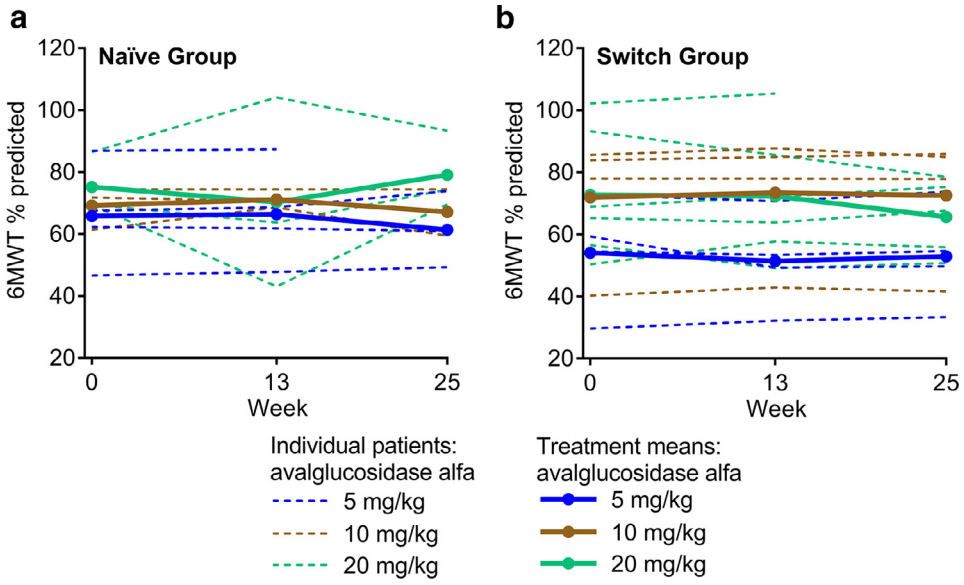

Fig. 7. 6-min walk test (6MWT) % predicted, (a) Naïve Group and (b) Switch Group.

Table S5 shows a summary of mean±SD, median, minimum, and maximum % predicted 6MWT distances at baseline and Week 25, along with changes from baseline at Week 25 for both groups by dose level). At baseline, during the 6MWT patients in the Naïve Group walked a mean±SD total distance of 449±118 (range 208–593) m and those in the Switch Group walked total distance of 440±141 (range 201–657) m. In the Naïve Group, mean±SD changes from baseline in 6MWT distances were 12.0±21.0 m, –15.3±15.5 m, and 24.3±23.0 m for the 5, 10, and 20 mg/kg groups, respectively, and for the Switch Group they were –4.0±30.5 m, 4.3±8.1 m, and –6.2±64.3 m, respectively.

3.7.3. GSGC test

For the Naïve and Switch Groups, mean changes from baseline at Week 25 for functional ability GSGC score remained unchanged relative to baseline at all doses, with overall means of –0.8 for the Naïve Group and 0.2 for the Switch Group.

The GSGC combines timed tests for walking 10 m, climbing four stairs, standing from sitting on the floor and standing from sitting on a chair. Although, the total score for these four tests did not change over the course of the study, test results for selected subtests showed mean decreases in both Naïve and Switch Group patients. The mean±SD changes from baseline for times to walk 10 m, climb four stairs, stand from sitting on the floor, and stand from sitting on a chair at Week 25 for the overall Naïve Group were –0.6±1.01 s, –1.1±2.21 s, –3.0±6.72 s, and –0.2±0.86 s, respectively, and for the overall Switch Group were –0.4±2.73 s, –0.5±2.83 s, 1.7±3.12 s, and –0.1±0.72 s, respectively.

3.7.3. GMFM-88 test

GMFM-88-DE scores for individual patients and treatment means are shown in Fig. 8A and B, for the Naïve and Switch Groups, respectively. For both groups, mean scores for Dimensions D (standing) and E (walking, running, and jumping), and the combined GMFM-88-DE functional strength total assessments remained unchanged relative to baseline in all treatment groups. The % mean±SD change from baseline for GMFM-88-DE score at Week 25 for the overall Naïve Group was 3.0±5.9% and for the overall Switch Group was 2.2±9.0%.

3.7.4. QMFT

QMFT scores for individual patients and treatment means are shown in Fig. 8C and D, for the Naïve and Switch Groups, respectively. In the Naïve Group, the mean±SD QMFT scores changed relative to baseline at Week 25 in the 5, 10, and 20 mg/kg groups by 0.7±4.9, 1.7±2.3, and 3.0±2.7 points, respectively, whilst in the Switch Group they changed by –1.5±2.7, 3.0±1.6, and 1.2±1.9 points, respectively.

3.7.5. HHD test

Lower body HHD scores for individual patients and treatment means are shown in Fig. 8E and F, for the Naïve and Switch Groups, respectively. In the Naïve Group, lower body HHD assessments changed at Week 25 relative to baseline by a mean±SD of 11.6±4.7%, 21.4±10.3%, and 14.2±15.9% in the 5, 10, and 20 mg/kg groups, respectively, whilst for the upper body assessments they changed by 8.2±25.9%, 19.0±7.5%, and –9.9±16.9%, respectively. In the Switch Group, lower body HHD assessments changed at Week 25 relative to baseline by –0.5±13.1%, 14.3±27.3%, and –14.5±42.2% in the 5, 10, and 20 mg/kg groups, respectively, whilst for the upper body assessments they changed by –8.1±24.5%, 10.8±17.8%, and –15.3±27.7%, respectively.

3.7.6. PedsQL

For both groups, mean scores for cognitive fatigue, general fatigue, and sleep/rest fatigue were mostly unchanged relative

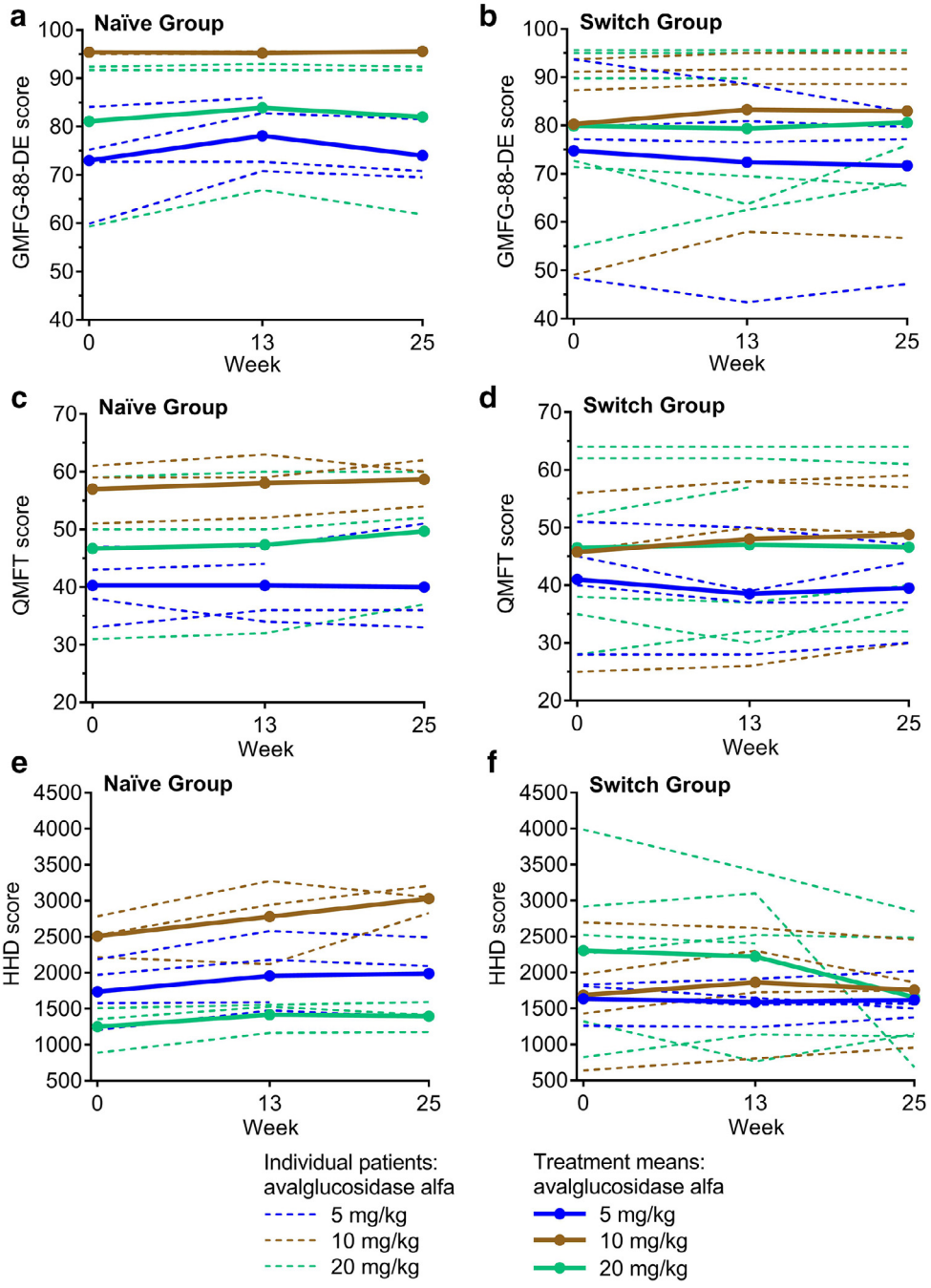

Fig. 8. Gross Motor Function Measure-88 for Dimensions D and E (GMFM-88-DE), Quick Motor Function Test (QMFT), and Hand-Held Dynamometry (HHD) sum of lower body for the Naïve Group (a, c, and e, respectively) and the Switch Group (b, d, and f, respectively).

to baseline. However, in the Naïve Group, there was a mean±SD increase in cognitive fatigue from baseline at Week 25 in the 5 mg/kg group (–13.9±6.4) and a decrease in cognitive fatigue and general fatigue (11.1±9.6 and 8.3±7.2, respectively) in the 20 mg/kg group. For the Switch Group, the two greatest increases in fatigue from baseline at Week 25 were for general fatigue in the 5 mg/kg group (–12.5±4.8) and for sleep/rest fatigue, also in the 5 mg/kg group (–6.2±4.2).

4. Discussion

In this open-label, ascending-dose study, avalglucosidase alfa was generally safe and well-tolerated at doses of 5, 10, and 20 mg/kg in adult patients with Pompe disease who were either treatment-naïve or had previously been treated with alglucosidase alfa for ≥9 months. No deaths or life-threatening SAEs were reported. Three treatment-emergent SAEs were reported by 2 patients. One Naïve Group

(5 mg/kg) patient reported two SAEs of respiratory distress and chest discomfort, both were considered study-drug related and led to study withdrawal. The other patient who reported a SAE was in the Switch Group (5 mg/kg) and experienced gastrointestinal hemorrhage, which was not considered study drug-related and did not lead to treatment discontinuation or study withdrawal. For both groups, the majority of TEAEs were mild across all dose levels. Across both groups, 25 IARs were reported in 8 (33%) patients.

Anti-avalglucosidase alfa antibodies were characterized because rhGAA studies suggest that antibodies to exogenous GAA may affect IARs [48] and/or efficacy in some LOPD patients [49]. Seroconversion for anti-avalglucosidase alfa antibodies was detected in the majority (90%) of Naïve Group patients and in 2 of 9 (22%) Switch Group patients, who had no anti-avalglucosidase alfa antibodies at baseline. Highest peak titers were 25,600 (1 Naïve Group patient) and 12,800 (1 Switch Group patient); the patient in the Naïve group improved on pulmonary and motor function testing and the patient in the Switch group improved on pulmonary function testing and declined on motor function testing. Titers for the remaining Naïve Group seroconverters remained ≤3200 and for the remaining Switch Group seroconverters remained ≤1600. No patients tested positive for enzyme activity inhibition, and only 1 patient, who was in the Naïve Group, tested positive for enzyme uptake inhibition. We note the presence of antibodies to avalglucosidase alfa at baseline in the Switch group, which may represent cross-reactivity with common epitopes from recombinant human GAA. Complement appeared to mediate IARs in 1 patient per group, although only 2 Naïve Group and 1 Switch Group patients were tested.

Avalglucosidase alfa plasma concentrations appeared to decline monoexponentially following the end of the infusion, with a mean $t_{½z}$ of ~1.0 h for both groups; consistent with avalglucosidase alfa non-clinical data, this was relatively shorter than observed for alglucosidase alfa (2.3 h) [25]. Avalglucosidase alfa pharmacokinetics appeared generally similar between groups and avalglucosidase alfa exposure increased with dose. The pharmacokinetic parameters appeared similar at Weeks 1, 13, and 25, indicating no apparent effect of every-other-week dosing on avalglucosidase alfa pharmacokinetics.

Baseline quadriceps muscle biopsy glycogen levels were generally low (~6% of tissue area) in both groups and remained mostly unchanged throughout the study. In addition, glycogen content is generally low in adults with Pompe disease and there is limited longitudinal information regarding glycogen clearance from muscle with enzyme replacement therapy. In the Exploratory Muscle Biopsy Assessment Study (EMBASSY) of alglucosidase alfa in LOPD [21], quadriceps biopsy glycogen was found both in lysosomes and cytoplasm at baseline (mean 5.3%; range 1.0–14.2%); after 24 weeks' therapy, lysosomal glycogen decreased in 10 patients and increased in 3 patients. Statistically significant changes were noted in 6 patients (4 had decreased glycogen and 2 increased glycogen), while cytoplasmic glycogen remained, and the total area of glycogen showed small reductions from baseline. Similarly, a study by Ripolone et al. [50] showed quadriceps biopsy improvements (e.g., fewer small Periodic Acid–Schiff (PAS)-positive glycogen accumulations and reduced severity of vacuolation, using different techniques than in EMBASSY or our study) in 15 of 18 LOPD patients after ≥6 months of alglucosidase alfa. Thus, biopsy results from our current avalglucosidase alfa study appear to be similar to alglucosidase alfa studies in LOPD.

Across both groups, urinary Hex_4 levels decreased at all avalglucosidase alfa doses (indicating reduction of glycogen burden), except for 5 mg/kg at Week 13 for the Switch Group. The trend for reduction was most pronounced in the Naïve group, similar to trends described in IOPD patients at initiation of enzyme replacement therapy [51]. There was no clear relationship to dose level. Of note, Hex_4 levels in LOPD prior to therapy are often not as high as those in IOPD [52], so that post-treatment trends may be less clear-cut in LOPD.

Muscle MRI at baseline indicated relatively mildly affected patients in both groups, which remained largely stable with little change throughout the 24-week treatment period. These results were similar to the EMBASSY study of alglucosidase alfa in LOPD [21], in which patients began with a mild degree of involvement and MRI parameters did not change materially during 6 months of treatment.

In both groups, pulmonary function generally improved or was stable at Week 25 relative to baseline in most patients. The evaluation of FVC % predicted is widely used as a pulmonary function endpoint in Pompe disease [53] and other neuromuscular diseases. In chronic respiratory diseases, FVC change over time is a valid measurement [54]. Repeated FVC % predicted measurement in untreated LOPD has revealed annual changes of −1.0% to −4.6% upright and 1.3% to −5.5% supine [54]. MIP and MEP have been evaluated in only four published papers on Pompe disease [55–58].

While efficacy assessments were purely exploratory, for both groups, patients remained stable or demonstrated improvements via several functional assessments. 6MWT distances were generally stable or tended to increase with avalglucosidase alfa, without a clear relationship to patient group or dose level. For both groups, GSGC and GMFM-88 assessments showed minimal changes, whereas improvements were observed in the QMFT and HHD evaluations. Decreases in cognitive and general fatigue were reported at 20 mg/kg avalglucosidase alfa by Naïve Group patients. Fatigue is salient in patients' experience of LOPD, with 2 out of 3 International Pompe Association survey respondents identifying it as among the three most disabling symptoms [59]. Decreased fatigue on avalglucosidase alfa therapy in naïve patients in our study parallels previous experience of reduced fatigue in alglucosidase alfa recipients [59,60].

The focus of this phase 1 study was safety, pharmacokinetic, and pharmacodynamic characteristics of avalglucosidase alfa in a small cohort of LOPD patients. Exploratory efficacy parameters demonstrated, in general, that pulmonary function remained stable or improved, as did functional capacity. The LOTS [28,29], a study

of alglucosidase alfa in children and adults with LOPD, demonstrated improvement in pulmonary function and distance walked after 78 weeks of therapy. It is therefore reassuring that functional and respiratory decline was not observed during this short period of treatment with avalglucosidase alfa.

Our study had some limitations. Firstly, a primary efficacy period of only 24 weeks precluded assessment of long-term treatment effects. Secondly, since the study cohort was small, with only 3–6 patients per dose group, it is difficult to draw firm conclusions from the data regarding efficacy. Finally, the design of the study does not allow comparison with previous safety or immunogenicity data from alglucosidase alfa studies that were placebo-controlled or open-label and not dose-escalating. The currently recruiting head-to-head study, COMET (NCT02782741), will directly compare the efficacy and safety of avalglucosidase alfa with alglucosidase alfa in LOPD patients not previously treated for Pompe disease with enzyme replacement therapy.

5. Conclusions

Avalglucosidase alfa had a well-tolerated safety profile in LOPD patients, either naïve to alglucosidase alfa therapy or who had previously received alglucosidase alfa therapy for ≥9 months. Whilst the efficacy assessments were exploratory, the results of this study support further development of avalglucosidase alfa.

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Data sharing statement

Qualified researchers may request access to patient level data and related study documents including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications. Patient level data will be anonymized and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://www.clinicalstudydatarequest.com/

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2018.12.004.

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