Molecular Genetics and Metabolism 119 (2016) 115–123

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Prospective exploratory muscle biopsy, imaging, and functional assessment in patients with late-onset Pompe disease treated with alglucosidase alfa: The EMBASSY Study

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ARTICLE INFO

Article history:
Received 1 March 2016
Received in revised form 16 May 2016
Accepted 17 May 2016
Available online 19 May 2016

Keywords:
Alglucosidase alfa
Enzyme replacement therapy
Functional effects
Glycogen
Histopathology
Late-onset Pompe disease
Muscle pathology
Pompe disease

ABSTRACT

Background: Late-onset Pompe disease is characterized by progressive skeletal myopathy followed by respiratory muscle weakness, typically leading to loss of ambulation and respiratory failure. In this population, enzyme replacement therapy (ERT) with alglucosidase alfa has been shown to stabilize respiratory function and improve mobility and muscle strength. Muscle pathology and glycogen clearance from skeletal muscle in treatment-naïve adults after ERT have not been extensively examined.

Methods: This exploratory, open-label, multicenter study evaluated glycogen clearance in muscle tissue samples collected pre- and post- alglucosidase alfa treatment in treatment-naïve adults with late-onset Pompe disease. The primary endpoint was the quantitative reduction in percent tissue area occupied by glycogen in muscle biopsies from baseline to 6 months. Secondary endpoints included qualitative histologic assessment of tissue glycogen distribution, secondary pathology changes, assessment of magnetic resonance images (MRIs) for intact muscle and fatty replacement, and functional assessments.

Results: Sixteen patients completed the study. After 6 months of ERT, the percent tissue area occupied by glycogen in quadriceps and deltoid muscles decreased in 10 and 8 patients, respectively. No changes were detected on MRI from baseline to 6 months. A majority of patients showed improvements on functional assessments after 6 months of treatment. All treatment-related adverse events were mild or moderate.

Conclusions: This exploratory study provides novel insights into the histopathologic effects of ERT in late-onset Pompe disease patients. Ultrastructural examination of muscle biopsies demonstrated reduced lysosomal glycogen after ERT. Findings are consistent with stabilization of disease by ERT in treatment-naïve patients with late-onset Pompe disease.

Abbreviations: 6MWT, 6-Minute Walk Test; AE, adverse event; BMI, body mass index; CI, confidence interval; ERT, enzyme replacement therapy; FVC, forced vital capacity; GAA, α-glucosidase; HRLM, high-resolution light microscopy; PFT, pulmonary function testing; GMFCS-E&R, Gross Motor Functional Classification System–Expanded and Revised; GMFM-88, Gross Motor Function Measure-88; GSGC, Gait, Stair, Gower's Maneuver, and Chair; LOTS, Late-Onset Treatment Study; MRI, magnetic resonance imaging; PedsQL, Pediatric Quality-of-Life Inventory; QMFT, Quick Motor Function Test.

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http://dx.doi.org/10.1016/j.ymgme.2016.05.013

1. Introduction

Pompe disease is a rare, autosomal recessive disorder caused by deficiency of lysosomal acid α-glucosidase (GAA), an enzyme that breaks down glycogen in the body [1]. The resulting lysosomal glycogen accumulation, especially in cardiac and skeletal muscle, disrupts muscle function leading to multisystem pathology, disability, and ultimately death [2–4]. The classic infantile form of Pompe disease is rapidly progressive, characterized by cardiomegaly, hypotonia, and death from cardiorespiratory failure in the first year of life [5–7].

Late-onset Pompe disease has a more varied disease course and later manifestation from childhood to adulthood characterized by slowly progressive skeletal myopathy but without the cardiomyopathy typical of infantile Pompe disease [8–13]. Late-onset Pompe disease usually presents with slowly progressive myopathy, predominantly of the proximal muscles in the trunk and pelvic and shoulder girdles, while the degree of respiratory muscle involvement is variable [9]. As skeletal and respiratory muscle weakness progresses, patients often need ambulatory and ventilator assistance. Respiratory failure is therefore a cause of significant morbidity and the most frequent cause of death [8,9,14–17].

Alglucosidase alfa (Lumizyme®/Myozyme®, Sanofi Genzyme, Cambridge, MA, USA) is an enzyme replacement therapy (ERT) for the treatment of Pompe disease that provides patients with exogenous recombinant human GAA [18–20]. In infantile-onset Pompe disease, alglucosidase alfa prolongs overall and ventilator-free survival and improves cardiomyopathy, motor skills, and functional independence [21,22]. In late-onset disease, alglucosidase alfa stabilizes respiratory function and improves mobility and muscle strength [23–25].

Muscle pathology and the pharmacodynamic effects of alglucosidase alfa in clearing glycogen from skeletal muscle have been examined in skeletal muscle biopsies from infantile Pompe patients [26]. Better response to treatment was observed in patients with early-stage cell damage at baseline characterized by predominance of lysosomal glycogen accumulation. Lesser response to treatment was seen in patients with more advanced disease characterized by predominance of cytoplasmic glycogen and ultrastructural damage [26]. Data on muscle histopathology and effects of ERT on glycogen clearance from skeletal muscle in patients with late-onset Pompe disease indicate that there is clinical heterogeneity and variable response to treatment among patients [27–31]. Additional morphologic studies examining pre- and post-ERT muscle biopsies are needed to help determine appropriate timing of treatment initiation for optimal responses for adult patients with Pompe disease. This exploratory study used muscle biopsies, magnetic resonance imaging (MRI) of skeletal muscle, and functional assessments to characterize disease burden and the effects of 6 months of alglucosidase alfa in treatment-naïve patients with late-onset Pompe disease. The results support the proposed biological activity of alglucosidase alfa and characterize its histopathological and functional effects in late-onset Pompe disease.

2. Methods

2.1. Study design

The Exploratory Muscle Biopsy Assessment Study (EMBASSY; NCT01288027, Sanofi Genzyme) was an open-label, multicenter study to evaluate glycogen clearance in muscle tissue samples and imaging assessments collected pre- and post-alglucosidase alfa treatment (20 mg/kg of body weight every other week for 6 months) in treatment-naïve late-onset Pompe disease patients. We also explored possible correlations between glycogen content, MRI, and functional assessments.

Eligible patients were ≥18 years of age with confirmed GAA enzyme deficiency from any tissue source and/or confirmed *GAA* gene mutations without known cardiac hypertrophy. The main inclusion criteria were the ability to walk 50 m without stopping and without an assistive device and forced vital capacity (FVC) in the upright position ≥50% predicted. Exclusion criteria were prior treatment with ERT, need for a wheelchair or invasive ventilation, and formal contraindication to MRI (e.g., pacemaker or implanted ferromagnetic metals).

2.2. Study assessments

The primary endpoint was the reduction in the percent tissue area occupied by glycogen in muscle biopsies from baseline to 6 months. The type of biopsy performed (open or needle) was chosen by the clinical sites based on individual laboratory capabilities and expertise. Biopsies performed at 6 months were performed on the same side of the body, near the original (baseline) site but far enough away so that there would not be any interference from scar tissue at the site of the baseline biopsy. Muscle biopsies were fixed in a glutaraldehyde-based fixative, embedded in epoxy resin, and processed for high-resolution light microscopy (HRLM) and electron microscopy as previously described [26,32]. Muscle glycogen content in HRLM sections was measured by computer morphometry and expressed as "percent tissue area occupied by glycogen" in quadriceps and deltoid muscle biopsies as previously described [32]. As this was an exploratory study, analyses were not blinded. Computer morphometry was used for objective analysis of glycogen. Serial sections from these epoxy resin blocks were prepared for electron microscopy and used to confirm, when necessary, the qualitative observations made on HRLM sections, such as localization of glycogen to the lysosomes or cytoplasm and presence of autophagic debris, fibrosis, and fatty replacement. When feasible, muscle MRI was used to guide the level (i.e., axial slice position) that the biopsy should target in order to capture the least-affected tissue (i.e., avoiding fatty replaced tissue). Secondary endpoints included qualitative histopathological assessment of biopsies, skeletal muscle imaging, and functional assessments.

Skeletal muscle MRI using qualitative T1-weighted imaging in all patients and quantitative T2 and Dixon modalities in a subset of patients was performed at baseline and 6 months. MRIs were read and analyzed by a central laboratory (C.R.I.S., Tournai, Belgium). The T1-weighted data were analyzed using Mercuri grading (1: normal appearance, 2: mild involvement, 3: moderate involvement, and 4: severe involvement) to determine the degree of intact muscle and fatty replacement. The Mercuri grading system provides a qualitative measure of disease involvement. Water T2 imaging provides a quantitative measure of disease activity (e.g., inflammation, sarcoplasmic leakage, cell edema, or necrosis) within muscles, where an abnormal value is defined as >39 milliseconds (ms). T2 determination based on multi spin-echo sequences typically requires knowledge of the radio-frequency transmitter field (B1) spatial deviation in the image, which typically requires an additional acquisition for computing the B1 maps. Muscle T2 can be estimated without this additional acquisition, but at the expense of a loss in precision. Therefore, T2 values (ms) are provided both with and without B1 sorting. In fatty infiltrated muscles, water T2 was separated from the fat signals by tri-exponential fitting of the global signal decay [33]. The percent of fatty infiltration in lower limb muscles was quantified using a 3-point 3D Dixon acquisition. For each subject, the average for each upper (thigh) and lower leg was computed for Mercuri grading, percentage of fat, muscle water, and T2 with and without B1 sorting. Muscle trophicity also was evaluated at the quadriceps level

by measuring the cross-sectional area of the four heads on three slices at mid-femur.

Functional efficacy was evaluated at baseline, 3 months, and 6 months using the following validated assessment tools: pulmonary function testing (PFT); 6-Minute Walk Test (6MWT); Quick Motor Function Text (QMFT), formerly the Rotterdam Motor Function Test; hand-held dynamometry; Gross Motor Function Measure-88 (GMFM-88); Gait, Stair, Gower's Maneuver, and Chair (GSGC); Pediatric Quality-of-Life Inventory (PedsQL) Multidimensional Fatigue Scale; and Gross Motor Functional Classification System–Expanded and Revised (GMFCS-E&R).

Treatment-emergent adverse events (AEs) were categorized by seriousness, severity, and whether the AE led to study discontinuation, and classified by the investigator as not related, unlikely related, possibly related, or related to the study drug or procedures.

2.3. Statistical analysis

This exploratory study was not powered to make statistical inferences. Formal sample size calculations were not performed. All patients who received at least one complete infusion of alglucosidase alfa were included in the analysis. A paired *t*-test was used to test for statistically significant absolute change from baseline in percent tissue area occupied by glycogen for each patient (the primary endpoint) and for the percent change in the primary endpoint. For the secondary endpoints, change from baseline to 3 months (if measured) and 6 months was calculated and a *t*-test was performed to estimate the 95% confidence interval (CI) and P value.

3. Results

3.1. Patient disposition and baseline characteristics

Between July 2011 and December 2013, 20 patients were screened for eligibility, of which 16 patients were enrolled at 11 sites in the United States, Germany, the Netherlands, and the United Kingdom. All 16 patients received 20 mg/kg alglucosidase alfa; all completed the study. Evaluable paired biopsy samples were available for the quadriceps muscle of 13 patients and deltoid muscle of 10 patients.

Baseline characteristics are shown in Table 1. Three patients used an assisted walking device. The mean distance walked on the baseline 6MWT was 450 m (range: 173–997 m). The mean percent predicted FVC was 76% (range: 50–115%) in the upright position and 57% (range: 27–119%) in the supine position. On the GMFCS-E&R assessment, 3 patients were Level I, 10 were Level II, and 3 were Level III. The mean T1-weighted Mercuri score at baseline was 1.9 (0.77) in the upper leg (n = 13) and 1.1 (0.27) in the lower leg (n = 14).

Table 1
Demographics and baseline patient characteristics.

Parameter	Patients receiving alglucosidase alfa (N = 16)
Age at study enrollment (y), mean (SD), median (min, max)	51.6 (13.69) 56.9 (24.5, 70.7)
Sex, n (%)	
Female	9 (56.3)
Male	7 (43.8)
Height (cm), mean (SD)	174.0 (12.08)
Weight (kg), mean (SD)	74.9 (17.40)
BMI (kg/m^2), mean (SD)	24.5 (4.01)
Age at first symptoms (y), mean (SD) (min, max)	40.0 (11.58) (14.4, 59.4)
Age at Pompe disease diagnosis (y), mean (SD) (min, max)	50.2 (13.48) (20.1, 66.1)

Abbreviations: BMI = body mass index; max = maximum; min = minimum; SD = standard deviation.

3.2. Tissue glycogen content

The type and location of muscle biopsy performed at baseline and 6 months are provided online (Online Supplementary Table 1). At baseline, total glycogen (lysosomal plus cytoplasmic glycogen) levels were generally higher in the quadriceps muscle (mean: 5.3%; range: 1.0–14.2%; n = 14) than in the deltoid muscle (mean: 2.4%; range: 1.2–5.9%; n = 12). As shown in Fig. 1A, the percent tissue area occupied by glycogen in the quadriceps showed a downward trend in 10 patients and increased in 3 patients. Statistically significant changes were noted in 6 patients (4 decreased glycogen and 2 increased glycogen). As shown in Fig. 1B, the percent tissue area occupied by glycogen in the deltoid showed a downward trend in 8 patients, increased in 1 patient, and was unchanged in 1 patient. Statistically significant decreases were noted in 5 patients.

In baseline biopsies, glycogen was present in a number of different locations across the cell. HRLM sections show glycogen-filled lysosomes as discrete, small, round PAS-positive structures (Fig. 2A, green arrows). Electron microscopy images confirm the lysosomal nature of these structures, 1–2 μm in diameter, and characterized by smooth, intact, round-to-oval membranes (Fig. 3A and C, green arrows). Glycogen was also present within the cytoplasm as thin, PAS-positive cytoplasmic streaks, as larger accumulations in cytoplasmic pools, and associated with central cores of autophagic debris (Fig. 2A). Free cytoplasmic glycogen also accumulated below the sarcolemmal membrane creating PAS-positive outer membrane blebs (Fig. 2B). Electron microscopy examination confirmed the cytoplasmic nature of glycogen in these areas (Figs. 3A–D).

Both HRLM (Fig. 2) and electron microscopic (Fig. 3) examination of post-treatment biopsies revealed a paucity of the small, intact, glycogen-filled lysosomes. Other features noted at baseline, such as free cytoplasmic glycogen present within streaks, pools, autophagic debris cores, and peripheral blebs, persisted in post-treatment biopsies. Because biopsy samples were embedded in epoxy resin to optimally preserve the glycogen for computer morphometry, acid-phosphatase staining and immunochemistry for other markers of autophagy could not be performed on tissue samples prepared this way. These investigations therefore were not part of this study. No fibrosis, inflammation, or fatty replacement were observed in biopsies, a finding potentially attributable to protocol instructions to biopsy muscle tissue that appeared normal as determined by MRI.

3.3. Skeletal muscle MRI

On T1-weighted MRI of the upper and lower leg muscle using Mercuri scoring, evaluable assessments at baseline and 6 months were available for the upper and lower leg in 13 and 14 patients, respectively. Overall, mean Mercuri scores indicated mild muscle involvement, varying from normal to moderate for individual patients, with the upper leg (1.9 ± 0.8) more affected than the lower leg (1.1 ± 0.3). Muscles within individual patients exhibited a heterogeneous pattern of involvement. In the thigh, adductor magnus, semi-membranous, semi-tendinous and long head of biceps femoris were the most infiltrated, while rectus femoris, gracilis and sartorius showed very little damage. Lower leg muscles were spared, with the exception of gastrocnemius medially, which occasionally presented very mild involvement. The tongue, subscapularis, and lumbar extensors were always affected, moderately to severely, confirming previous descriptions [34]. At 6 months, overall changes from baseline in mean Mercuri scores were not observed in either the upper leg (mean change: 0.0 [95% CI: −0.0, 0.1]) or lower leg (mean change: 0.0 [95% CI: 0.0, 0.0]). Quantitative comparison of the degree of fatty infiltration from baseline to 6 months using 3-point 3D Dixon imaging was possible in 5 patients. Percentages of fatty infiltration were normal or elevated (>10%) in accordance with the Mercuri scoring degree. Overall, no changes from baseline were apparent at 6 months (mean change: 0.6 [95% CI: −1.7, 3.0]; P = 0.49). Muscle

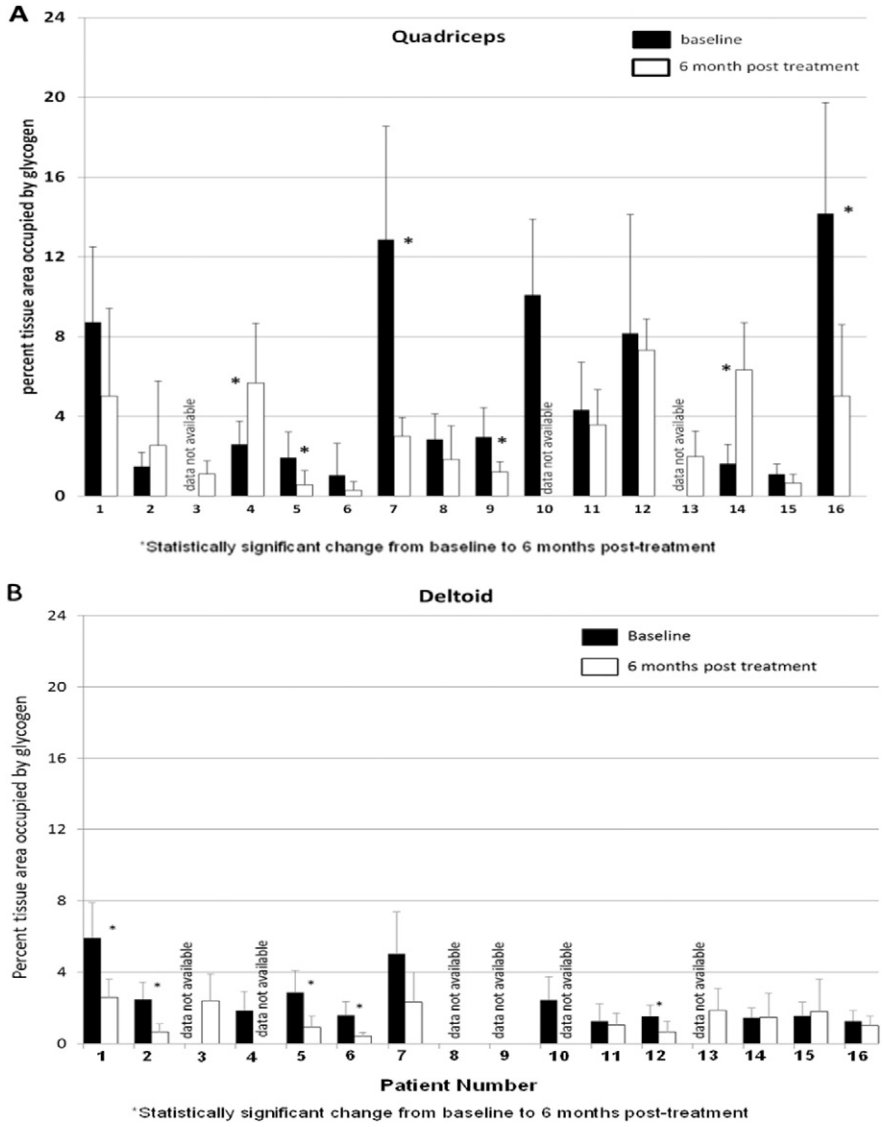

Fig. 1. MetaMorph analysis of glycogen content in quadriceps and deltoid biopsies, pre- and post-treatment. Up to 10 sections derived from multiple tissue blocks from each biopsy were imaged digitally, analyzed by computer morphometry and expressed as percent tissue area occupied by glycogen. Analyses were not blinded, and computer morphometry provided objective analysis of glycogen. The values from these sections were averaged to obtain a mean and standard deviation at each patient-time point. This approach thereby takes into account any variability in the distribution of glycogen across the entire biopsy. There was an overall trend toward reduction or stabilization of glycogen levels in post-treatment biopsies. Total glycogen load in deltoid samples were consistently lower than that measured in quadriceps biopsies. Panel A: quadriceps biopsy samples. Panel B: deltoid biopsy samples.

water T2, a non-specific marker of disease activity that senses mainly edema and inflammation, could be quantified at baseline in 9 patients with and 13 patients without B1 sorting.

Approximately one-third of all muscles had abnormally elevated T2 muscle water at baseline. After 6 months, there were no obvious water T2 MRI changes compared with baseline for analysis with B1 (mean change: 2.2 ms [95% CI: −0.9, 5.2]; $P = 0.15$) or without B1 (mean change: 1.9 ms [95% CI: −0.5, 4.3]; $P = 0.10$). Quadriceps cross-sectional area revealed a modest (2%) but significant increase in muscle mass (mean change: 106 mm^2; $P = 0.05$).

3.4. Functional assessments

Functional improvements were observed from baseline to 6 months (Table 2). The mean percent predicted upright FVC changed from 76.4% to 77.6% (mean absolute increase: 1.8 percentage points; $P = 0.67$). Statistically significant improvements at 6 months were seen on QMFT, where the mean score improved by 5.3% ($P = 0.04$; $n = 15$), and on the 6MWT, where the mean distance walked increased by 37 m ($P = 0.02$; $n = 15$).

3.5. Exploratory correlation analyses

Exploratory analyses were conducted to assess potential correlations among glycogen clearance and the functional assessments. Reduced quadriceps muscle glycogen content correlated significantly with improved outcomes on QMFT ($r = -0.8426$, $P = 0.004$) but did not correlate with increased distance walked on 6MWT ($r = -0.4231$, $P = 0.26$) in all patients. Among 10 patients with paired data for comparison of within-patient reduction in quadriceps glycogen content with knee extensor muscle strength (where quadriceps is the main force) on the biopsied side, there was no change in muscle strength. However, there was glycogen clearance suggesting that enzyme enters the lysosomes.

3.6. Safety outcomes

There were no AEs leading to study withdrawal and no deaths. There was one serious AE, which was not related to treatment. All treatment-related AEs (24 events in 6 [35.5%] patients) were mild or moderate in severity. Four (25%) patients experienced infusion-associated reactions.

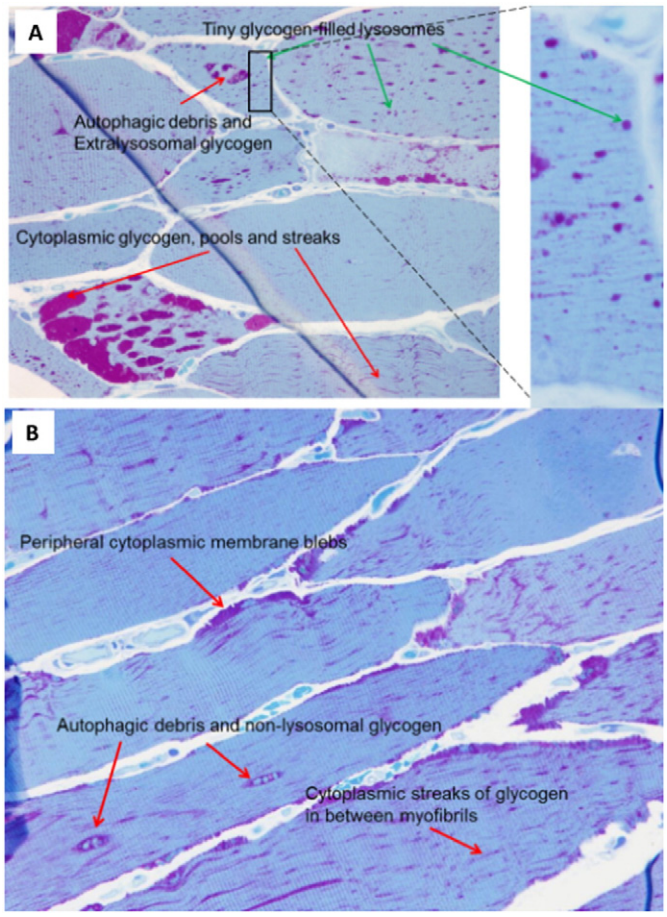

Fig. 2. Pre- and post-treatment biopsy findings by high-resolution light microscopy (HRLM). Biopsy samples shown are from the quadriceps muscles of the same patient (Patient 12). Features that appear responsive to ERT are noted with green arrows. Features that persist after ERT are noted with red arrows. Panel A: In baseline biopsy samples, glycogen was located intra-lysosomally in small, round PAS positive lysosomes (green arrows; see high magnification insert). Glycogen was also present within the cytoplasm, not bound within lysosomal membranes, but floating freely in between myofibrils. These areas appear as thin, PAS-positive cytoplasmic streaks in HRLM sections (red arrows). In areas with myofibrillar damage, the cytoplasmic glycogen accumulates further, displacing the myofibrils, and appears as larger, PAS-positive pools (red arrow). Glycogen was also associated with centrally located cores of autophagic debris (red arrow). At the periphery of some myocytes, free glycogen accumulates beneath the outer cell membrane and appears as PAS-positive blebs (not shown in baseline image; see similar structure in panel B). Panel B: Post-treatment biopsy samples revealed a paucity of the small, intact, glycogen-filled lysosomes. The other features noted at baseline (free cytoplasmic glycogen present within streaks, pools, autophagic debris cores, and peripheral blebs) persisted in post-treatment biopsies. These HRLM features of pre- and post-treatment biopsy samples were confirmed by electron microscopy of serial sections. (HRLM, 1 μm epoxy resin section, PAS and Richardson's stain, 400× magnification).

4. Discussion

This study provides the first prospective evidence of the histopathologic effects of alglucosidase alfa in late-onset Pompe disease patients. Baseline biopsies identified glycogen present within lysosomes and as free cytoplasmic glycogen. Alglucosidase alfa treatment reduced lysosomal glycogen, with the glycogen remaining after 6 months of treatment being predominantly cytoplasmic (i.e., extra-lysosomal). Non-lysosomal-bound glycogen has also been shown to persist in post-treatment biopsies of Pompe infants [26]. Because the enzyme is taken up by receptor-mediated endocytosis via the mannose-6-phosphate receptor, alglucosidase alfa relies on endosomal delivery of the enzyme via fusion with intact lysosomes and has optimal activity within this acidic microenvironment. We observed qualitative decreases in small intact lysosomes, which is consistent with this mechanism of action. Further study is needed to confirm and validate this observation. Glycogen blebs that are free floating within the cytoplasm are therefore presumably inaccessible to the enzyme and thus remain. Safety outcomes were consistent with previous studies of alglucosidase alfa in patients with late-onset Pompe disease [25,35].

This study illustrates the similarities and differences between infantile and late-onset pathology and disease progression. Histologic glycogen accumulation observed in infants was 5–10 times higher by histomorphometry measurement than in the adult biopsies here, reflecting the rapidly progressive nature of the infantile disease, which exhibits global immobility shortly after birth by disturbance of the contractile apparatus of skeletal muscle [6,26,36,37]. In adults, this process seems to be much slower with mobility deficits emerging gradually over time [36]. MRI studies show the earliest disease occurring in the paraspinal and trunk muscles and eventually the thigh muscles [34], as well as a selective pattern of muscle damage with trunk involvement even in asymptomatic patients [38]. In adults, muscle groups located in regions of the body that are subject to more continuous or repetitive contraction (e.g., axial skeletal muscles for standing, lower limb muscles for ambulation, diaphragm for breathing) appear to manifest clinical decline and histologic damage earlier in the disease compared with muscle groups that are subject to relatively intermittent contraction (e.g., deltoid within the upper limbs), which manifest clinical deterioration later [9,12]. This suggests that the differences in frequency and intensity of the biomechanical forces of contraction in different muscle groups may determine the evolution of lysosomal disruption, cytoplasmic glycogen accumulation, ultrastructural damage, and the rate of clinical disease progression within these muscles. This may also explain the higher baseline glycogen levels observed in quadriceps biopsies compared with deltoid biopsies. Further additional analyses of different fiber types are needed to confirm or refute this hypothesis.

Our analysis indicates that 6 months of alglucosidase alfa treatment is apparently sufficient to show an effect on lysosomal glycogen clearance in most adults. Our findings are consistent with muscle biopsy findings obtained at baseline and 3 and 12 months in a clinical study of 8 infantile Pompe disease patients, in which the extent of glycogen clearance varied widely with some samples showing dramatic glycogen reduction and others showing further accumulation [26]. In both infants and adults, the glycogen that remained refractory to treatment was extra-lysosomal, suggesting that treatment is most effective when the disease process in the individual muscle fibers is not yet too far advanced [39], while the cellular mechanisms to degrade both lysosomal and cytoplasmic glycogen properly are still intact and are not yet disturbed or are still working properly. This was illustrated by the fact that the post-treatment biopsies revealed a paucity of the small, intact, glycogen-filled lysosomes. The other features noted at baseline (free cytoplasmic glycogen present within streaks, pools, autophagic debris cores, and peripheral blebs) persisted in post-treatment biopsies. The effect of ERT, therefore, cannot be quantified. The underlying pathologic mechanism responsible for the persistence of pools and streaks of free cytoplasmic glycogen not amendable to therapy is not fully understood. However, if it is the result of lysosomal rupture, then it suggests that ERT may have been initiated too late in this group of patients. The results underscore the importance of additional studies focusing on the timing of initiation of ERT in adults with Pompe disease.

Muscle MRI and functional disease classification indicated a relatively mildly affected patient cohort that remained largely stable throughout the study. The baseline overall Mercuri scores showed mild muscle involvement, varying from normal to moderate for individual patients. Despite the low incidence and extent of chronic degenerative changes, it is worth noting that, when water T2 could be measured, approximately one-third of muscles had abnormally elevated T2, similar to an observation recently reported in another group of adult Pompe

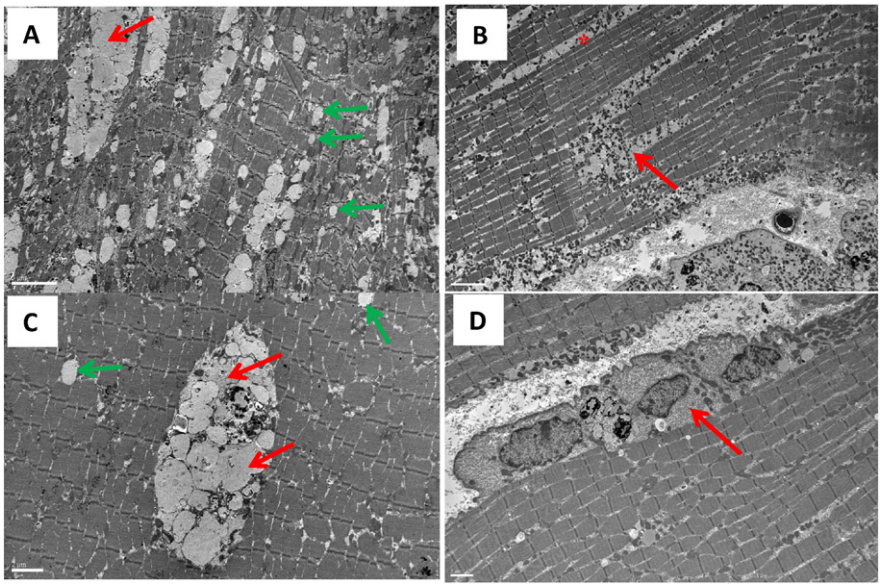

Fig. 3. Intracellular glycogen localization was confirmed by electron microscopic examination. Biopsy samples are from the quadriceps muscle of the same patient (Patient 12). Features that appear responsive to ERT are noted with green arrows. Features that persist after ERT are noted with red arrows. Panel A: Glycogen-filled lysosomes, 1–2 μm in diameter, are present singly in between myofibrils (green arrows). Glycogen that has escaped lysosomes and floats freely in the cytoplasm merges to form an early pool of glycogen (red arrow). The tattered, discontinuous nature of the membranes suggests disruption of lysosomal integrity and release of glycogen into the cytoplasm. Panel B: In early streaks, glycogen floats freely in between myofibrils (red asterisk). As more glycogen accumulates within the cytoplasm, myofibrillar structure is disrupted (red arrow). Panel C: Glycogen was also associated with centrally located cores of autophagic debris. In these areas, glycogen can be observed spilling out of disrupted lysosomes and into the cytoplasm. These disrupted lysosomes are characterized by broken, tattered and discontinuous membranes (red arrows). Two intact lysosomes are visible in the periphery (green arrows). Panel D: Free cytoplasmic glycogen pushed to the periphery of the cell accumulates beneath the outer sarcolemmal membrane, forming periphery blebs which appear PAS positive on HRLM (see Fig. 2B). Scale bars indicate magnification in each panel.

Table 2
Pulmonary and physical function from baseline to 6 months.

	Baseline	6 months	Change from baseline	Percent change from baseline
Forced vital capacity (L), % predicted				
Upright (n = 15)	76.4 (15.63) (50.4, 115.1)	77.6 (28.46) (49.3, 174.8)	1.8 (16.39) (−7.3, 10.9) P = 0.67	0.4 (15.03) (−7.9, 8.8) P = 0.91
Supine (n = 14)	57.0 (24.84) (26.9, 118.7)	60.8 (34.09) (25.7, 161.3)	2.9 (12.35) (−4.5, 10.4) P = 0.41	1.5 (12.23) (−5.9, 8.9) P = 0.67
Maximum Inspiratory Pressure ($cm H_2O$), % predicted				
Upright (n = 13)	64.0 (29.58)	65.6 (28.25)	1.6 (13.22) (−6.4, 9.6) P = 0.68	4.4 (27.18) (−12.0, 20.8) P = 0.57
Supine (n = 6)	55.2 (35.11)	58.1 (32.57)	−8.2 (12.32) (−21.1, 4.8) P = 0.17	−14.7 (27.58) (−43.7, 14.2) P = 0.25
Maximum Expiratory Pressure ($cm H_2O$), % predicted				
Upright (n = 13)	69.2 (27.43)	71.6 (29.98)	2.4 (14.88) (−6.6, 11.4 P = 0.57	1.3 (24.02) (−13.2, 15.8) P = 0.85
Supine (n = 6)	49.0 (27.78)	53.2 (36.79)	−11.6 (12.25) (−24.4, 1.3) P = 0.07	−30.0 (29.97) (−61.4, 1.4) P = 0.06
6-Minute Walk Test (n = 15)	449.9 (208.01) (173.0, 997.0)	471.2 (223.60) (139.0, 1007.0)	37.3 (53.55) (7.7, 67.0) P = 0.02	7.9 (12.91) (0.8, 15.1) P = 0.03
Quick Motor Function Test (n = 15)	44.5 (11.87) (26.0, 64.0)	46.8 (12.31) (24.0, 64.0)	Not reported	5.3 (9.37) (0.1, 10.5) P = 0.045
Hand-held dynamometry – upper body (n = 15)	2065.5 (859.85) (1589.3, 2541.7)	2108.7 (850.70) (1637.6, 2579.8)	43.2 (272.80) (−107.8, 194.3) P = 0.55	4.7 (20.46) (−6.7, 16.0) P = 0.39
Hand-held dynamometry – lower body (n = 15)	1764.0 (795.67) (1323.4, 2204.6)	1952.3 (839.40) (1487.4, 2417.1)	188.3 (405.42) (−36.2, 412.8) P = 0.09	18.7 (45.89) (−6.7, 44.1) P = 0.14
Gross Motor Function Measure-88 (n = 16)	84.0 (20.17) (24.1, 100.0)	86.7 (17.46) (40.0, 100.0)	Not reported	6.1 (16.30) (−2.6, 14.8) P = 0.16
Gait, Stairs, Gower's Maneuver, and Chair (n = 16)	13.4 (5.19) (5.0, 21.0)	12.5 (6.04) (4.0, 23.0)	−0.9 (2.22) (−2.1, 0.3) P = 0.14	Not reported
Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (n = 15)	58.5 (20.74) (12.5, 87.5)	66.0 (15.73) (36.1, 91.7)	8.1 (17.01) (−1.4, 17.5) P = 0.088	Not reported

Baseline and 6-month values are mean (standard deviation) (min, max).
Change and percent change data are mean (standard deviation) (95% confidence interval) P value.

patients [40]. In these patients, muscles with higher water T2 experienced faster progression of fatty degenerative changes. This strongly suggests that elevated water T2 can reveal subclinical damage in muscle of still-normal appearance. The confirmation of a high percentage of muscles with abnormal water T2 in Pompe patients brings in a new element of reflection for early therapeutic intervention. The functional disease measure GMFCS-E&R, also indicated mild overall functional impairment. Both at the cohort level and for most individual patients, this clinical disease classification was consistent with the MRI-based Mercuri scores.

Although alglucosidase alfa improves walking distance and respiratory function in late-onset Pompe disease [23,25,35], skeletal muscle weakness has been shown to persist [41]. Patients in our study were able to walk a mean 37 m farther on the 6MWT and had a mean increase of 1.8 percentage points in percent predicted FVC in the upright position. These functional improvements are generally consistent with the longer, double-blind, placebo-controlled Late-Onset Treatment Study (LOTS), where the increase distance walked on the 6MWT at 18 months was 25 m and the increase in percent predicted upright FVC was 1.2 percentage points [25], despite baseline differences between our 16 patients and the 90 patients in LOTS. Compared with alglucosidase alfa-treated patients in LOTS, on average, our patients were 10 years older at symptom onset, 6 years older at first infusion, had been living with Pompe disease 2 years longer, and had percent predicted upright FVC 20 points higher [25]. Interestingly, a small but significant increase in quadriceps trophicity was detected after 6 months of ERT, which is similar to an observation made some years ago [42]. The increase in muscle mass might have contributed to the gain in 6MWT or this increase may have been the consequence of improved physical activity with ERT.

Examining glycogen clearance in muscle biopsies of ERT-treated adults with late-onset Pompe disease is important for understanding the apparent resistance of skeletal muscle to ERT. In our study, the reduction in quadriceps glycogen content for individual patients was significantly associated with motor function improvement on the QMFT ($r = -0.84$; $P = 0.004$). However, baseline glycogen content in muscle biopsies correlated poorly with skeletal muscle imaging and functional indicators of disease severity at baseline. This is likely due to sampling and pathology analysis of a single muscle belly, whereas imaging and functional assessments evaluate the disease and function of a group of muscle bellies acting in concert. Thus, muscle biopsy assessment is useful in answering proof-of-concept questions and evaluating disease state and therapeutic response of a single muscle belly, but may be less reflective of the musculoskeletal system as a whole. Biopsy sampling of multiple muscles would be ideal, but a practical impossibility. Whole body MRI of Pompe patients would best evaluate the stage of disease in multiple muscles, but systematic quantitative imaging and generation of parametric maps of percentage of muscle fat content and muscle water T2 will allow more meaningful correlations with clinical functional outcomes.

This uncontrolled, exploratory study was not statistically powered to demonstrate significant changes from baseline. It was designed to demonstrate proof-of-concept in adults and to explore changes in muscle histopathology underlying the clinical and functional improvements associated with alglucosidase alfa observed in LOTS [25,35].

Nonetheless, these muscle biopsy results in adults with late-onset Pompe disease demonstrate reduction of lysosomal glycogen in response to alglucosidase alfa treatment, with maintenance of cytoplasmic glycogen, within a relatively short treatment period of 6 months. This treatment effect observed at the histopathologic level was accompanied by improvement and/or stabilization in most clinical disease parameters, which is consistent with stabilization of disease by alglucosidase alfa in treatment-naïve adults with late-onset Pompe disease.

Informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (international and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients included in this analysis.

Author contributions

BLT, PGC and CB designed the study. BLT and PGC performed data acquisition and statistical analyses. PGC, BLT, and CB analyzed and interpreted the results. BLT and CB wrote the manuscript. All authors interpreted the results and critically reviewed early and final drafts of the manuscript. BLT, PGC and CB take responsibility for the accuracy and integrity of the manuscript.

Conflicts of interest

AvP: served as an advisor, participated in various clinical trials and received research grants under agreements between Erasmus MC University Medical Center and Sanofi Genzyme.

PGC: received honoraria for lectures; participated in an advisory board meeting and had expenses related to this participation reimbursed by Sanofi Genzyme.

RYC: received honoraria for a lecture in the 2nd Fabry Summer School and participated in an advisory board meeting and was reimbursed for related expenses for these participations by Sanofi Genzyme.

JTK: received research support for clinical trials in Pompe disease from Sanofi Genzyme and from Biomarin.

BS: received honoraria, consulting fees, and travel reimbursement for his work on the scientific advisory boards for Sanofi Genzyme; Biomarin Pharmaceutical Inc.; and Audentes Therapeutics.

SW: has no conflicts of interests or financial disclosures to report.

AP: received research funding from the NIH, Muscular Dystrophy Association, Neuromuscular Research Fund, Insmed, Knopp, Cytokinetics, Biogen Idec, ISIS Pharmaceuticals (now Ionis Pharmaceuticals), Sanofi Genzyme, GSK, and Ultragenyx.

RJB: is a member of speakers' bureaus for Sanofi Genzyme and Grifols and advisory boards for MedImmune and Novartis; received consulting fees and honoraria from Alexion, Isis, Baxter, Serepta, and CSL Behring; and received research funding from Cytokinetics, Inc., Sangamo, Knopp, Biogen, Neuraltus, TEVA, Sanofi Genzyme, Biomarin, PTC Therapeutics, the NIH, FDAOPD, and NINDS.

MMD: is a consultant for Sanofi Genzyme and has received grants from, or participated, in research studies funded by Amicus, Biomarin, and Sanofi Genzyme.

OGA: is a principle investigator and has received grant support and/or is on the speaker bureau for Sanofi Genzyme, Shire, Pfizer, Alexion, and Amicus.

TM: serves on the global advisory board for Pompe disease (Amicus, Biomarin, and Sanofi Genzyme), GNE (Ultragenyx), dermatomyositis (Idera) and sIBM (Novartis) and received travel subsidies and honoraria for related activities; has served as a consultant to NuFactor, Sarepta Therapeutics, and Walgreens and received travel subsidies and honoraria; served on the speaker's bureau for Sanofi Genzyme (Pompe disease) and Grifols (CIDP) and received travel subsidies and honoraria for these speaker's bureau activities; received research funding as a principal investigator or sub-investigator from the following sources: Alexion, Alnylam, Amicus, Baxter, Bio-Blast, Biogen, Biomarin, CSL Behring, Sanofi Genzyme, Grifols, GSK, Idera, ISIS Pharmaceuticals (now Ionis Pharmaceuticals), NIH, Novartis, and Ultragenyx.

LDMP: received speakers fees and honoraria for advisory board participation from Sanofi Genzyme and Avexis honoraria for advisory board participation.

ZS: received fees as a consultant for Cytokinetics, Inc. for ALS; received payment from Neuralstem, Inc. for serving on the Safety Monitoring Board of an ALS stem cell study; receives a yearly stipend from Wiley for services as a Senior Associate Editor for *Muscle & Nerve*.

VS: is, or has been, a principal investigator for trials sponsored by Sanofi Genzyme; GSK; Prosensa/Biomarin; ISIS Pharmaceuticals (now Ionis Pharmaceuticals); and Sarepta. He received speaker honoraria from Sanofi Genzyme and has been on advisory boards for Acceleron Pharma, Audentes Therapeutics, Bristol-Myers Squibb, Sanofi Genzyme, Italfarmaco S.p.A., NicOx, Pfizer, Prosensa, Santhera, Summit Therapeutics and TrophyNOD. He has a research collaboration with Ultragenyx and Sanofi Genzyme.

MG: has no conflicts of interests or financial disclosures to report.

PY: received consulting fees and honoraria for talks within the past 5 years by Sanofi Genzyme, Biomarin, UCB, Heinen und Löwenstein, RedMed, Teva, and Pharnext.

MB: received speaker honoraria, consulting fees, travel reimbursements and research funding from Genzyme GmbH, Neu-Isenburg, Germany, and Sanofi Genzyme.

PYB: has no conflicts of interests or financial disclosures to report.

SW: has no conflicts of interests or financial disclosures to report.

RS: is an employee of Sanofi Genzyme.

CB: is an employee of Sanofi Genzyme.

BLT: is an employee of Sanofi Genzyme.

Acknowledgments

This study was sponsored and conducted by Sanofi Genzyme (NCT01288027). We thank the laboratories in the Department of Pathology at Sanofi Genzyme for their expertise in lysosomal disease pathology; Laurie LaRusso of Chestnut Medical Communications for manuscript writing assistance paid for by Sanofi Genzyme; and Adrienne A. Aiello and Marianne B. Zajdel of Sanofi Genzyme for medical writing and editorial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2016.05.013.

References

[1] H.G. Hers, alpha-Glucosidase deficiency in generalized glycogenstorage disease (Pompe's disease), Biochem. J. 86 (1963) 11–16.
[2] A.G. Engel, M.R. Gomez, M.E. Seybold, E.H. Lambert, The spectrum and diagnosis of acid maltase deficiency, Neurology 23 (1973) 95–106.
[3] R. Hirschhorn, A.J. Reuser, Glycogen storage disease type II: acid α-glucosidase (acid maltase) deficiency, in: D. Valle, B. Vogelstein, K.W. Kinzler, S.E. Antonarakis, A. Ballabio, K. Gibson, G. Mitchell (Eds.), OMMBD: The Online Metabolic and Molecular Bases of Inherited Disease, McGraw-Hill, New York, 2014 (Available at http://ommbid.mhmedical.com/content.aspx?bookid=971&Sectionid=62641992),.
[4] A.T. van der Ploeg, A.J. Reuser, Pompe's disease, Lancet 372 (2008) 1342–1353.
[5] B.W. Adam, E.M. Hall, M. Sternberg, T.H. Lim, S.R. Flores, S. O'Brien, D. Simms, L.X. Li, V.R. De Jesus, W.H. Hannon, The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States, Clin. Biochem. 44 (2011) 1445–1450.
[6] P.S. Kishnani, W.L. Hwu, H. Mandel, M. Nicolino, F. Yong, D. Corzo, Infantile-Onset Pompe Disease Natural History Study Group, A retrospective, multinational, multicenter study on the natural history of infantile-onset Pompe disease, J. Pediatr. 148 (2006) 671–676.
[7] H.M. van den Hout, W. Hop, O.P. van Diggelen, J.A. Smeitink, G.P. Smit, B.T. Poll-The, H.D. Bakker, M.C. Loonen, J.B. de Klerk, A.J. Reuser, A.T. van der Ploeg, The natural course of infantile Pompe's disease: 20 original cases compared with 133 cases from the literature, Pediatrics 112 (2003) 332–340.
[8] ACMG, Work Group on Management of Pompe Disease, P.S. Kishnani, R.D. Steiner, D. Bali, K. Berger, B.J. Byrne, L.E. Case, J.F. Crowley, S. Downs, R.R. Howell, R.M. Kravitz, J. Mackey, D. Marsden, A.M. Martins, D.S. Millington, M. Nicolino, G. O'Grady, M.C. Patterson, D.M. Rapoport, A. Slonim, C.T. Spencer, C.J. Tifft, M.S. Watson, Pompe disease diagnosis and management guideline, Genet. Med. 8 (2006) 267–288.
[9] E.J. Cupler, K.I. Berger, R.T. Leshner, G.I. Wolfe, J.J. Han, R.J. Barohn, J.T. Kissel, AANEM Consensus Committee on Late-onset Pompe Disease, Consensus treatment recommendations for late-onset Pompe disease, Muscle Nerve 45 (2012) 319–333.
[10] M.L. Hagemans, A.C. Janssens, L.P. Winkel, K.A. Sieradzan, A.J. Reuser, P.A. Van Doorn, A.T. Van der Ploeg, Late-onset Pompe disease primarily affects quality of life in physical health domains, Neurology 63 (2004) 1688–1692.
[11] M.L. Hagemans, L.P. Winkel, W.C. Hop, A.J. Reuser, P.A. Van Doorn, A.T. Van der Ploeg, Disease severity in children and adults with Pompe disease related to age and disease duration, Neurology 64 (2005) 2139–2141.
[12] P. Laforet, M. Nicolino, P.B. Eymard, J.P. Puech, C. Caillaud, L. Poenaru, M. Fardeau, Juvenile and adult-onset acid maltase deficiency in France: genotype-phenotype correlation, Neurology 55 (2000) 1122–1128.
[13] N.A. van der Beek, O.I. Soliman, C.I. van Capelle, M.L. Geleijnse, W.B. Vletter, M.A. Kroos, A.J. Reuser, I.M. Frohn-Mulder, P.A. van Doorn, A.T. van der Ploeg, Cardiac evaluation in children and adults with Pompe disease sharing the common c.-32-13T>G genotype rarely reveals abnormalities, J. Neurol. Sci. 275 (2008) 46–50.
[14] D. Gungor, J.M. de Vries, W.C. Hop, A.J. Reuser, P.A. van Doorn, A.T. van der Ploeg, M.L. Hagemans, Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy, Orphanet J. Rare Dis. 6 (2011) 34.
[15] D. Gungor, M.E. Kruijshaar, I. Plug, R.B. D'Agostino, M.L. Hagemans, P.A. van Doorn, A.J. Reuser, A.T. van der Ploeg, Impact of enzyme replacement therapy on survival in adults with Pompe disease: results from a prospective international observational study, Orphanet J. Rare Dis. 8 (2013) 49.
[16] M.L. Hagemans, W.J. Hop, P.A. Van Doorn, A.J. Reuser, A.T. van der Ploeg, Course of disability and respiratory function in untreated late-onset Pompe disease, Neurology 66 (2006) 581–583.
[17] N.A. van der Beek, J.M. de Vries, M.L. Hagemans, W.C. Hop, M.A. Kroos, J.H. Wokke, M. de Visser, B.G. van Engelen, J.B. Kuks, A.J. van der Kooi, N.C. Notermans, K.G. Faber, J.J. Verschuuren, A.J. Reuser, A.T. van der Ploeg, P.A. van Doorn, Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study, Orphanet J. Rare Dis. 7 (2012) 88.
[18] Lumizyme® (Alglucosidase Alfa) for Injection, for Intravenous Use. [Prescribing Information], Cambridge, MA: Genzyme Corporation, 2009 (Revised August 2014).
[19] Myozyme® (Alglucosidase Alfa) Injectable for Intravenous Infusion. [Prescribing Information], Cambridge, MA: Genzyme Corporation, 2009 (Revised May 2014).
[20] M. Nicolino, Alglucosidase alfa: first available treatment for Pompe disease, Therapy 4 (2007) 271–277.
[21] P.S. Kishnani, D. Corzo, M. Nicolino, B. Byrne, H. Mandel, W.L. Hwu, N. Leslie, J. Levine, C. Spencer, M. McDonald, J. Li, J. Dumontier, M. Halberthal, Y.H. Chien, R. Hopkin, S. Vijayaraghavan, D. Gruskin, D. Bartholomew, A. van der Ploeg, J.P. Clancy, R. Parini, G. Morin, M. Beck, G.S. De la Gastine, M. Jokic, B. Thurberg, S. Richards, D. Bali, M. Davison, M.A. Worden, Y.T. Chen, J.E. Wraith, Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease, Neurology 68 (2007) 99–109.
[22] M. Nicolino, B. Byrne, J.E. Wraith, N. Leslie, H. Mandel, D.R. Freyer, G.L. Arnold, E.K. Pivnick, C.J. Ottinger, P.H. Robinson, J.C. Loo, M. Smitka, P. Jardine, L. Tato, B. Chabrol, S. McCandless, S. Kimura, L. Mehta, D. Bali, A. Skrinar, C. Morgan, L. Rangachari, D. Corzo, P.S. Kishnani, Clinical outcomes after long-term treatment with alglucosidase alfa in infants and children with advanced Pompe disease, Genet. Med. 11 (2009) 210–219.
[23] L.J. Anderson, W. Henley, K.M. Wyatt, V. Nikolaou, S. Waldek, D.A. Hughes, R.H. Lachmann, S. Logan, Effectiveness of enzyme replacement therapy in adults with late-onset Pompe disease: results from the NCS-LSD cohort study, J. Inherit. Metab. Dis. 37 (2014) 945–952.
[24] A. Toscano, B. Schoser, Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review, J. Neurol. 260 (2013) 951–959.
[25] A.T. van der Ploeg, P.R. Clemens, D. Corzo, D.M. Escolar, J. Florence, G.J. Groeneveld, S. Herson, P.S. Kishnani, P. Laforet, S.L. Lake, D.J. Lange, R.T. Leshner, J.E. Mayhew, C. Morgan, K. Nozaki, D.J. Park, A. Pestronk, B. Rosenbloom, A. Skrinar, C.I. van Capelle, N.A. van der Beek, M. Wasserstein, S.A. Zivkovic, A randomized study of alglucosidase alfa in late-onset Pompe's disease, N. Engl. J. Med. 362 (2010) 1396–1406.
[26] B.L. Thurberg, C. Lynch Maloney, C. Vaccaro, K. Afonso, A.C. Tsai, E. Bossen, P.S. Kishnani, M. O'Callaghan, Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease, Lab. Investig. 86 (2006) 1208–1220.
[27] C. Angelini, C. Semplicini, S. Ravaglia, B. Bembi, S. Servidei, E. Pegoraro, M. Moggio, M. Filosto, E. Sette, G. Crescimanno, P. Tonin, R. Parini, L. Morandi, G. Marrosu, G. Greco, O. Musumeci, G. Di Iorio, G. Siciliano, M.A. Donati, F. Carubbi, M. Ermani, T. Mongini, A. Toscano, G.G. Italian, Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years, J. Neurol. 259 (2012) 952–958.
[28] L. Maggi, F. Salerno, C. Bragato, S. Saredi, F. Blasevich, E. Maccagnano, B. Pasanisi, C. Danesino, M. Mora, L. Morandi, Familial adult-onset Pompe disease associated with unusual clinical and histological features, Acta Myol. 32 (2013) 85–90.
[29] M. Sciacco, D. Ronchi, M. Ripolone, R. Violano, V. Lucchini, R.G.P. Xhani, G.P. Comi, F. Fortunato, A. Bordoni, P. Tonin, M. Filosto, S. Previtali, T. Mongini, L. Vercell, E. Vittonatto, A. Toscano, O. Musumeci, E. Barca, C. Angelini, C. Lamperti, M. Mora, L. Morandi, M. Moggio, Late-onset Pompe disease: histopathological, biochemical and clinical assessment before and after ERT, Neuromuscul. Disord. 24 (2014) 869.
[30] C.I. van Capelle, L.P. Winkel, M.L. Hagemans, S.K. Shapira, W.F. Arts, P.A. van Doorn, W.C. Hop, A.J. Reuser, A.T. van der Ploeg, Eight years experience with enzyme replacement therapy in two children and one adult with Pompe disease, Neuromuscul. Disord. 18 (2008) 447–452.
[31] L.P. Winkel, J.M. Van den Hout, J.H. Kamphoven, J.A. Disseldorp, M. Remmerswaal, W.F. Arts, M.C. Loonen, A.G. Vulto, P.A. Van Doorn, G. De Jong, W. Hop, G.P. Smit, S.K. Shapira, M.A. Boer, O.P. van Diggelen, A.J. Reuser, A.T. Van der Ploeg, Enzyme replacement therapy in late-onset Pompe's disease: a three-year follow-up, Ann. Neurol. 55 (2004) 495–502.

[32] C.M. Lynch, J. Johnson, C. Vaccaro, B.L. Thurberg, High-resolution light microscopy (HRLM) and digital analysis of Pompe disease pathology, J. Histochem. Cytochem. 53 (2005) 63–73.
[33] N. Azzabou, P. Loureiro de Sousa, E. Caldas, P.G. Carlier, Validation of a generic approach to muscle water T2 determination at 3T in fat-infiltrated skeletal muscle, J. Magn. Reson. Imaging 41 (2015) 645–653.
[34] R.Y. Carlier, P. Laforet, C. Wary, D. Mompoint, K. Laloui, N. Pellegrini, D. Annane, P.G. Carlier, D. Orlikowski, Whole-body muscle MRI in 20 patients suffering from late onset Pompe disease: involvement patterns, Neuromuscul. Disord. 21 (2011) 791–799.
[35] A.T. van der Ploeg, R. Barohn, L. Carlson, J. Charrow, P.R. Clemens, R.J. Hopkin, P.S. Kishnani, P. Laforet, C. Morgan, S. Nations, A. Pestronk, H. Plotkin, B.E. Rosenbloom, K.B. Sims, E. Tsao, Open-label extension study following the Late-Onset Treatment Study (LOTS) of alglucosidase alfa, Mol. Genet. Metab. 107 (2012) 456–461.
[36] B.G. Schoser, J. Muller-Hocker, R. Horvath, K. Gempel, D. Pongratz, H. Lochmuller, W. Muller-Felber, Adult-onset glycogen storage disease type 2: clinico-pathological phenotype revisited, Neuropathol. Appl. Neurobiol. 33 (2007) 544–559.
[37] L.P. Winkel, J.H. Kamphoven, H.J. van den Hout, L.A. Severijnen, P.A. van Doorn, A.J. Reuser, A.T. van der Ploeg, Morphological changes in muscle tissue of patients with infantile Pompe's disease receiving enzyme replacement therapy, Muscle Nerve 27 (2003) 743–751.
[38] A. Alejaldre, J. Diaz-Manera, S. Ravaglia, E.C. Tibaldi, F. D'Amore, G. Moris, N. Muelas, J.J. Vilchez, A. Garcia-Medina, M. Uson, F.A. Martinez Garcia, I. Illa, A. Pichiecchio, Trunk muscle involvement in late-onset Pompe disease: study of thirty patients, Neuromuscul. Disord. 22 (Suppl. 2) (2012) S148–S154.
[39] N. Raben, E. Ralston, Y.H. Chien, R. Baum, C. Schreiner, W.L. Hwu, K.J. Zaal, P.H. Plotz, Differences in the predominance of lysosomal and autophagic pathologies between infants and adults with Pompe disease: implications for therapy, Mol. Genet. Metab. 101 (2010) 324–331.
[40] P.G. Carlier, N. Azzabou, P.L. de Sousa, A. Hicks, J.M. Boisserie, A. Amadon, R.Y. Carlier, C. Wary, D. Orlikowski, P. Laforet, Skeletal muscle quantitative nuclear magnetic resonance imaging follow-up of adult Pompe patients, J. Inherit. Metab. Dis. 38 (2015) 565–572.
[41] S. Strothotte, N. Strigl-Pill, B. Grunert, C. Kornblum, K. Eger, C. Wessig, M. Deschauer, F. Breunig, F.X. Glocker, S. Vielhaber, A. Brejova, M. Hilz, K. Reiners, W. Muller-Felber, E. Mengel, M. Spranger, B. Schoser, Enzyme replacement therapy with alglucosidase alfa in 44 patients with late-onset glycogen storage disease type 2: 12-month results of an observational clinical trial, J. Neurol. 257 (2010) 91–97.
[42] S. Ravaglia, A. Pichiecchio, M. Ponzio, C. Danesino, K. Saeidi Garaghani, G.U. Poloni, A. Toscano, A. Moglia, A. Carlucci, P. Bini, M. Ceroni, S. Bastianello, Changes in skeletal muscle qualities during enzyme replacement therapy in late-onset type II glycogenosis: temporal and spatial pattern of mass vs. strength response, J. Inherit. Metab. Dis. 33 (2010) 737–745.