Effect of alglucosidase alfa dosage on survival and walking ability in patients with classic infantile Pompe disease: a multicentre observational cohort study from the European Pompe Consortium

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Summary

Background Enzyme replacement therapy (ERT) with alglucosidase alfa has been found to improve outcomes in patients with classic infantile Pompe disease, who without treatment typically die before the age of 1 year. Variable responses to the standard recommended dosage have led to alternative dosing strategies. We aimed to assess the effect of real-world ERT regimens on survival and walking ability in these patients.

Methods In this observational cohort study, we obtained data collected as part of a collaborative study within the European Pompe Consortium on patients with classic infantile Pompe disease from France, Germany, Italy, and the Netherlands diagnosed between Oct 26, 1998 and March 8, 2019. Eligible patients had classic infantile Pompe disease with a disease onset and proven diagnosis before age 12 months, and a hypertrophic cardiomyopathy. A proven diagnosis of classic infantile Pompe disease was defined as a confirmed deficiency of α-glucosidase in leukocytes or lymphocytes, fibroblasts or muscle, or two pathogenic GAA variants in trans, or both. We collected data on demographics, GAA variants, ERT dosage, age at death, and walking ability. We analysed the effects of ERT dosage on survival and walking ability using Cox regression, Kaplan-Meier curves, and log-rank tests.

Findings We included 124 patients with classic infantile Pompe disease, of whom 116 were treated with ERT (median age at start of treatment 3·3 months [IQR 1·8–5·0, range 0·03–11·8]). During follow-up (mean duration 60·1 months [SD 57·3]; n=115), 36 (31%) of 116 patients died. 39 different ERT dosing regimens were applied. Among the 64 patients who remained on the same dosage, 16 (52%) of 31 patients on the standard dosage (20 mg/kg every other week), 12 (80%) of 15 patients on an

intermediate dosage (20 mg/kg per week or 40 mg/kg every other week), and 16 (89%) of 18 patients on the high dosage (40 mg/kg per week) were alive at last follow-up. Survival was significantly improved in the high dosage group compared with the standard dosage group (hazard ratio [HR] 0·17 [95% CI 0·04–0·76], p=0·02). No significant difference in survival was identified between the intermediate dosage group and the standard dosage group (HR 0·44 [0·13–1·51], p=0·19). Of the 86 patients who reached 18 months of age, 44 (51%) learned to walk. Ten (53%) of 19 patients on the standard dosage regimen, six (67%) of nine patients on intermediate dosage regimens, and 14 (93%) of 15 patients on high dosage regimens learnt to walk, but the differences between groups were not statistically significant.

Interpretation Patients with classic infantile Pompe disease treated with the high ERT dosage of 40 mg/kg per week had significantly improved survival when compared with patients treated with the standard recommended ERT dosage of 20 mg/kg every other week. Based on these results, we suggest that the currently registered dosage should be reconsidered.

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Research in context

Evidence before this study

We searched PubMed, Embase, MEDLINE Ovid, Web of Science, and Cochrane from database inception to June 25, 2021, for studies reporting on clinical outcome in patients with classic infantile Pompe disease treated with α-glucosidase (enzyme replacement therapy [ERT]). We used the search terms "GSD II", "Pompe", "Pompe disease", "acid maltase deficiency", "Enzyme Replacement Therapy", "ERT", "α-glucosidase", "alpha-glucosidase", "acid maltase", "alglucosidase alfa", "alglucosidase alpha", and "Myozyme". Previous studies showed that patients with classic infantile Pompe disease can experience clinical deterioration despite treatment with the standard recommended dosage of alglucosidase alfa (20 mg/kg every other week). This finding has led to the use of different dosing strategies. To date, no large studies assessing the effects of ERT dosing on clinical outcomes in patients with classic infantile Pompe disease have been published.

Added value of this study

In this study, we collected real-world data from 116 patients with classic infantile Pompe who had received ERT, which, to our knowledge, is the largest cohort described to date. We provided evidence that patients treated with high dosage alglucosidase alfa (40 mg/kg per week) had improved survival when compared with patients treated with the standard recommended dosage (20 mg/kg every other week). The proportion of patients who learned to walk was also highest in the high dosage group.

Implications of all the available evidence

A plethora of ERT regimens are used to treat classic infantile Pompe disease in real-world clinical practice, as many patients experience clinical deterioration when given the standard recommended dosage. On the basis of our results, we suggest that the current standard recommended dosage of alglucosidase alfa in patients with classic infantile Pompe disease should be reconsidered. International data collection on additional clinical outcome measures such as ventilator-free survival, pulmonary function, motor function, cardiac function, neurocognitive function, cross-reactive immunological material status and antibody titre, and additional treatment with immunomodulation is needed for further treatment optimisation and implementation of future therapies in classic infantile Pompe disease.

Introduction

Pompe disease, which is also known as glycogen storage disease type 2 (Online Mendelian Inheritance in Man number 232300), is a progressive lysosomal storage dis- ease and neuromuscular disorder with autosomal recessive inheritance. The disease is caused by a deficiency of the enzyme acid α-glucosidase (GAA; Enzyme Commission number 3.2.1.20). The clinical spectrum is broad and continuous since symptoms can present in infancy, childhood, and adulthood.1 The classic infantile form of the disease is characterised by a progressive hypertrophic cardiomyopathy and generalised muscle weakness, which prevent children from achieving developmental motor milestones such as rolling over or walking. Without treatment, infants with classic infantile Pompe disease typically die from cardiorespiratory failure before the age of 1 year.2 Enzyme replacement therapy (ERT) with intravenous recombinant human α-glucosidase (alglucosidase alfa) has led to improved survival and motor outcomes.1, 3–6 Additionally, ERT results in a normalisation of cardiac hypertrophy and cardiac function in almost all patients with classic infantile Pompe disease.3–8

The first attempts at developing ERT for Pompe disease were made in the 1960s. Patients received enzyme preparations from human placenta and Aspergillus Niger.1 These treatments were not effective due to insufficient dosage, heterologous sources, or inappropriate glycosylation with absence of the mannose-6-phosphate group, which is necessary for adequate cellular uptake and transport to the lysosome.9,10 Cloning of the GAA gene11 enabled large-scale production of human recombinant α-glucosidase. Preclinical studies showed that the uptake of α-glucosidase is dose and tissue dependent, and that high doses were needed in Pompe disease to reach the skeletal muscles.12–14 The first trial in humans was done using recombinant human GAA from the milk of transgenic rabbits in four patients with classic infantile Pompe disease in 1999.3 These patients were initially treated with 15 mg/kg or 20 mg/kg per week, which was later increased to 40 mg/kg per week for all infants.3,4 The dosage of 40 mg/kg per week resulted in normalisation of α-glucosidase activity in muscle biopsies and improved clearance of glycogen. In subsequent studies with recombinant α-glucosidase obtained from Chinese hamster ovarian cells,15–17 the administered dosage varied from 5 mg/kg twice per week to 50 mg/kg per week (10 mg/kg given five times a week), and in the AGLU 01602 study,5 dosages of 20 or 40 mg/kg every other week were used. In 2006, ERT with recombinant human GAA from Chinese hamster ovarian cells was approved by the US Food and Drug Administration (FDA) and European Medicines Agency for use in all patients with Pompe disease. In the AGLU 01602 study, no significant differences with regard

to treatment efficacy were identified between the dosages of 20 and 40 mg/kg every other week, 5 thus 20 mg/kg every other week was registered as the standard treatment regimen.

However, since approval of alglucosidase alfa by the FDA and European Medicines Agency the optimum dosage for ERT in patients with classic infantile Pompe disease has remained a topic of debate.18 Several countries have published data on treatment outcomes in classic infantile Pompe disease. Survival in these studies ranged from 57% to 100%, and 25–88% of patients learnt to walk, with population sizes ranging from eight to 33 patients and follow-up durations ranging from 3·6 months to 13·7 years.7,8,19–22 However, only one of these studies compared implemented dosing strategies.8 During the long-term follow-up of this study, patients with classic infantile Pompe disease treated with 40 mg/kg per week continuously had better survival and motor outcome than those treated with 20 mg/kg every other week.22 On the basis of the findings of the first clinical trial3,4 from 2009 onwards, all patients in the Netherlands were treated with 40 mg/kg per week from treatment initiation.22 A comparative study with follow-up of at least 3 years showed improved outcomes in patients treated with 40 mg/kg per week when compared with the standard registered dosage of 20 mg/kg every other week.8 Similar findings were reported in an additional study.23 Another open label study also suggested an improved outcome after dosage elevation in patients with Pompe disease who experienced a clinical decline on standard treatment (20 mg/kg every other week).24 The attempts to optimise the effects of ERT have led to different dosing strategies in patients with classic infantile Pompe disease. However, to date, no large cohort studies comparing treatment dosage in patients with classic infantile Pompe disease have been published.18 Thus, we conducted a collaborative study with the European Pompe Consortium to assess the effect of real world ERT regimens on treatment outcomes (overall survival and walking ability).

Methods

Study design and participants

In this observational cohort study, we report data collected as part of a collaborative study within the European Pompe Consortium. The collaborative consortium consists of experts from 11 European countries with long-standing experience in treating and following up patients with Pompe disease. Data for untreated patients were not documented routinely in all countries, but we have reported all available data here separately to illustrate the difference between treated and untreated patients. Several patients in this cohort have been described in previous studies.3,4,7,8,19,21,22,25

We included all patients with classic infantile Pompe disease who were diagnosed between Oct 26, 1998 and March 8, 2019 in Germany, France, Italy, and the Netherlands. Eligible patients had a disease onset and diagnosis before the age of 12 months and a hypertrophic cardiomyopathy. Additionally, patients had a proven diagnosis of classic infantile Pompe disease, defined as a confirmed deficiency of α-glucosidase in leukocytes or lymphocytes, fibroblasts or muscle, or two pathogenic GAA variants in trans, or both.26 A hypertrophic cardio- myopathy was defined as a left ventricular mass value larger than 42 g and a left ventricular mass index value larger than 84 g/m², according to the Boston Children's Hospital Z score system.27 To be defined as a treated patient in this study, patients were required to have started treatment with alglucosidase alfa within the first year of life. Patients were only included in the walking analysis if they reached age 18 months during follow-up, because this is the age at which children with normal development should typically be able to walk.28

This study was approved by the medical ethics committees of the participating hospitals in the Netherlands and Italy, and medical ethics approval was obtained nationally in Germany and France and expanded to enable the use of data internationally. Informed consent was obtained from all participants' caregivers in this study.

Data collection

We collected de-identified data on the following: demographics and diagnosis (country, date of birth, sex, date of symptom onset, date of diagnosis, date of last follow-up visit, date and cause of death if applicable); cardiac parameters (presence of a hypertrophic cardiomyopathy, left ventricular mass index, left ventricular mass, body surface area, and Z score of the left ventricular mass index), treatment (ERT [yes or no], age at start of treatment, dosage regimen, changes in dosage regimen, reason for

changes in dosage, discontinuation of treatment, and reason for dis- continuation if applicable); and treatment outcome (survival and walking ability). With regard to the diagnosis of Pompe disease, we also collected data on GAA variants and the cross-reactive immunological material (CRIM) status of patients. Most patients with classic infantile Pompe disease produce some endogenous inactive GAA protein and are therefore considered CRIM-positive; the remainder of patients do not produce any detectable GAA protein and are considered CRIM-negative.

Due to the large variety in reported treatment regimens, we divided the regimens into two broad categories; constant and modified treatment regimens. Constant regimens were defined as treatment regimens in which the dosage of ERT remained the same from the start: standard dosage (20 mg/kg every other week), intermediate dosage (20 mg/kg per week or 40 mg/kg every other week), or high dosage (40 mg/kg per week). Modified regimens were defined as treatment regimens that were changed during follow-up. Within the modified regimen category, we defined three subcategories: increased dosage (regimen in which ERT dosage was increased), decreased dosage (regimen in which ERT dosage was decreased), and variable (variable combinations of dosage increases and decreases).

Statistical analysis

Descriptive analyses were performed by tabulating demographic data, clinical information, and different dose-frequency combinations of ERT. We used Fisher's exact test and Kruskal-Wallis tests to compare CRIM status and age at initiation of ERT for the constant treatment regimens. We used Kaplan-Meier survival analysis and log-rank tests to determine differences in survival from birth and the ability to walk for different dose-frequency combinations. Differences in treatment outcome (survival and walking ability) between patients who received intermediate or high dosage ERT were compared with the standard dosage and analysed using Cox regression. Proportional hazard assumptions were not violated. We made no adjustments for multiple comparisons. A p value of less than 0·05 was considered to indicate a statistically significant difference. Statistical analyses were done using SPSS (version 25.0) and GraphPad Prism (version 5.0).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

We included 124 patients with classic infantile Pompe disease diagnosed between Oct 26, 1998 and March 8, 2019, of whom 116 were treated with ERT (table 1). To our knowledge, all patients with classic infantile Pompe diagnosed during this time period in the four participating countries were included. 95 (82%) of the 116 patients started ERT after registration by the European Medicines Agency and the US FDA in 2006. Patients who started treatment between 1999 and 2001 were initially treated with recombinant human α-glucosidase from the milk of transgenic rabbits. Eight patients were not started on ERT. Mean follow-up duration was 60·1 months (SD 57·3) for the 115 patients who received ERT (one patient was excluded from the analysis due to a discrepancy in the data) and 2·1 (2·1) for the eight patients who did not receive ERT. The overall median age at symptom onset was 1·2 months (IQR 0·1–2·7) and median age at diagnosis was 3 months (1·5–4·6).

	Treated patients (n=116)	Untreated patients (n=8)
Country		
Germany	41 (35%)	0
France	19 (16%)	3 (38%)
Italy	30 (26%)	0
Netherlands	26 (22%)	5 (63%)
Sex		
Male	58 (50%)	6 (75%)
Female	58 (50%)	2 (25%)
Age at symptom onset, months	1·1 (0·1–2·6)	3·2 (1·2–4·4)
Age at diagnosis, months	3·0 (1·2–4·5)	4·6 (3·4–5·9)

Table 1: Baseline characteristics of all study participants (n=124) Data are n (%) or median (IQR). ERT=enzyme replacement therapy.

Among the 124 patients, pathogenic variants in the GAA gene were identified on 214 alleles. For 101 patients, a pathogenic variant was identified on both alleles of the GAA gene, and in one of these patients, three patho-genic variants were identified (two variants on one allele). In eight patients, only one pathogenic variant was identified. In three patients, one pathogenic variant and a protein change in GAA were reported. In two patients, only the two protein changes were reported, but the GAA variants were not stated. In ten patients, only enzyme assays were performed. In total, 78 different GAA pathogenic variants were identified, eight of which have not been reported before: 1030G→A, 1064C→T, 1130del, 1557G→A, 1571A→G, 1597T→C, 1714C→T, and 2337G→A. The second variant was identified for all eight patients, with the exception of the patient with the 1714C→T variant. A

subdivision was made according to CRIM-status (proven or predicted). The most frequently reported CRIM-negative variant was 525del (27 [13%] of 214 alleles) and the most frequently reported CRIM-positive variant was deletion of exon 18 (2481+102_2646+31del; 20 [9%] of 214 alleles). An overview of all individual combinations of GAA variants found in the study is provided in the appendix (pp 4,5).

ERT was started in 116 patients at a median age of 3·3 months (IQR 1·8–5·0, range 0·03–11·8). A summary of the dosage regimens used is in the appendix (p 1). 64 (55%) of 116 treated patients received the same dosage from the start until the end of the observation period, 31 (27%) received the standard dosage (20 mg/kg every other week), 15 (13%) received an intermediate dosage of 20 mg/kg per week or 40 mg/kg every other week, and 18 (16%) received a high dosage of 40 mg/kg per week during follow-up (table 2). Among 49 (42%) of 116 patients, the dosage of ERT was changed during follow-up: dosage was increased in 28 (24%) patients, decreased in five (4%) patients, and was variable (increased and decreased) in 15 (13%) patients; for one patient, the starting dosage was 20 mg/kg every other week, which was changed to 30 mg/kg with unknown frequency (appendix p 1). The exact ERT dosage regimen was unknown for four (3%) of 116 patients, one of whom was excluded from some analyses by treatment subgroup because of missing data. Of the 116 treated patients, 48 (41%) started ERT at a higher dosage than the standard recommended dosage (18 high dosage, 15 intermediate dosage, four variable dosage, four decreased dosage, and seven increased dosage). Severe disease state, higher dosages being deemed more effective, evidence from the literature, personal experience of the clinician, deterioration of the patient, insufficient response, respiratory insufficiency, late diagnosis, and standard practice were stated as reasons for prescribing dosages higher than the standard dosage. In five patients ERT dosage was decreased during study follow-up due to good response to treatment, insufficient response to treatment, or parents' decision.

	Overall	Standard dosage	Intermediate dosage	High dosage	Increased dosage	Decreased dosage	Variable dosage	Unknown
Number of patients	116	31	15	18	28	5	15	4
Age at ERT initiation, months	3·3 (1·8–5·0; n=115)	3·0 (1·3–4·5)	3·5 (1·8–5·0)	3·9 (2·7–5·0)	3·2 (1·3–5·7)	2·6 (2·5–7·1)	3·1 (2·0–5·2)	4·2 (3·1–NC*; n=3†)
Follow-up duration, months	60·1 (57·3; n=115)	41·9 (44·1)	48·8 (57·3; n=14‡)	59·6 (39·9)	77·5 (75·8)	91·2 (82·3)	72·1 (47·5)	38·9 (35·8)
Death	36	15	3	2	8	2	3	3
Age at death, months	22·3 (10·8–48·9)	16·1 (8·5–37·8)	29·6 (8·5–NC*)	21·8 (6·3–NC*)	24·6 (19·3–44·8)	126·1 (66·1–NC*)	17·7 (12·1–NC*)	30·5 (8·6–NC*)

Table 2: Treatment received, follow-up duration, and deaths Data are n, median (IQR), or mean (SD). NC=not calculable. ERT=enzyme replacement therapy. *The third quartile of the IQR could not be calculated because of small numbers of participants in these groups. †For one patient in the unknown dosage group, the age at ERT initiation was unknown, therefore the patient was excluded from the analysis of age at ERT initiation. ‡There was a discrepancy between data that could not be resolved for one patient in the intermediate group with regard to follow-up duration, therefore this patient was excluded from the analysis of follow-up duration.

To establish whether there was a significant difference in distribution of CRIM-status between constant ERT regimens, we did a Fisher's exact test. The standard regimen was most frequently prescribed (table 3), but no association was identified between constant ERT regimens and CRIM status (Fisher's exact test p=0·35). All eight untreated patients died within 11 months from birth, and the median age at death was 6·1 months (IQR 5·0–8·3). Causes of death included sudden cardiac arrest, progressive heart failure, and respiratory insufficiency. In one patient, the cause of death was not documented. Of the 116 treated patients, 36 (31%) patients died during follow-up. The median age at death was 22·3 months (IQR 10·8–48·9, range 3·9–186·2), and over- all survival is shown in figure 1A. Among the 64 patients who received a constant dosage of ERT, 16 (52%) of 31 patients on the standard dosage (20 mg/kg every other week), 12 (80%) of 15 patients on an intermediate dosage (20 mg/kg per week or 40 mg/kg every other week), and 16 (89%) of 18 patients on the high dosage (40 mg/kg per week) were alive at last follow-up (figure 1B). A significant difference in 5-year survival was identified between standard dosage and high dosage ERT (Z=2·97, log rank p=0·003). The difference in survival between standard dosage and intermediate dosage at 5 years was not statistically significant (Z=0·63, log rank p=0·53).

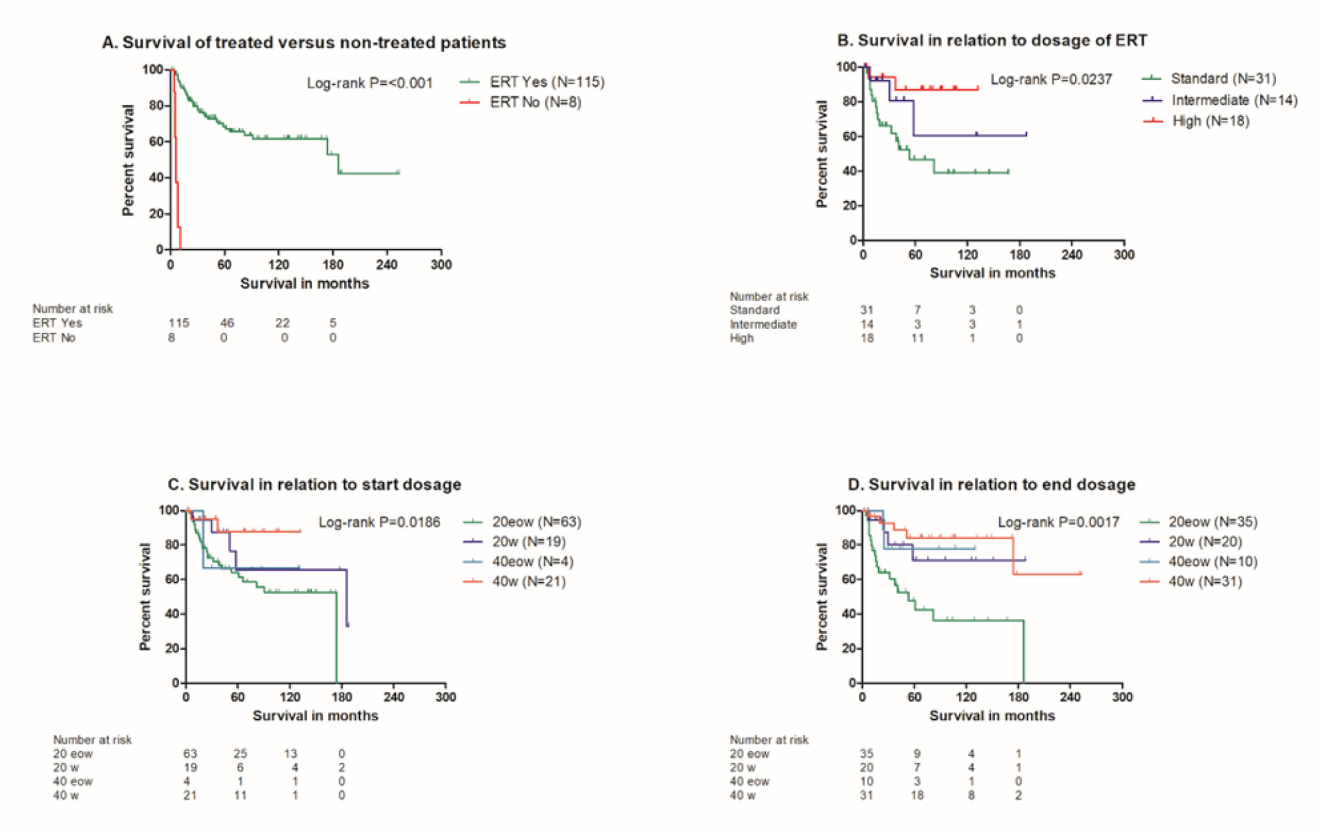

Figure 1: Kaplan-Meier overall survival by ERT status and ERT dosage Overall survival by ERT treatment status (A), ERT dosage in patients who received constant regimens (B), initial ERT dosage (C), and final ERT dosage (D). One patient had missing data on follow-up duration and was therefore excluded from the Kaplan-Meier analysis. In part A, data of the eight untreated patients are also provided for comparison. ERT=enzyme replacement therapy. 20eow= 20 mg/kg/every other week, 20w= 20 mg/kg/week, 40 eow=40 mg/kg/every other week, 40w= mg/kg/week.

	Constant regimens			**Modified regimens**			**Unknown regimen (n=4)**
	Standard dosage (n=31)	Intermediate dosage (n=15)	High dosage (n=18)	Increased dosage (n=28)	Decreased dosage (n=5)	Variable dosage (n=15)	
CRIM positive	19	10	14	18	0	8	3
CRIM negative	10	2	3	3	2	4	0
Unknown	2	3	1	7	3	3	1

Table 3: CRIM status by enzyme replacement therapy regimen Data are n. CRIM=cross-reactive immunological material.

Overall survival was significantly longer in the high dosage group than the standard dosage group (hazard ratio [HR] 0·17 [95% CI 0·04–0·76], p=0·02). No statistically significant difference in survival was identified between the intermediate dosage and standard dosage groups (0·44 [0·13–1·51], p=0·19). Due to the limited number of patients the analysis was not adequately powered to correct for other confounders in the Cox model (only 20 events occurred in the constant regimen groups). The minimal number of events per coefficient is 10; therefore, only two coefficients were added to the model (intermediate and high dosage). When including CRIM status in the Cox model, notwithstanding this model being less statistically stable, a similar effect of dosage on survival was observed. A statistically significant improvement in survival remained for the high dosage group when compared with the standard dosage group (HR 0·19 [95% CI 0·04–0·83], p=0·03). No statistically significant differences in age at ERT initiation were identified between the constant regimen treatment groups (Kruskal-Wallis p=0·40).

Since not all patients had the same starting and final dosage, we generated two survival curves regarding starting ERT dosage and final ERT dosage (figure 1C, D). 20 (71%) of 28 patients treated with increased dosage regimens, 12 (80%) of 15 patients treated with variable dosage regimens, and three (60%) of five patients treated with decreased dosage regimens were alive at last follow- up (appendix p 2). Cox regression analysis was not possible for these groups due to heterogeneity of the regimens. Survival was highest for patients who started on high dosage ERT, and lowest for patients who started on standard dosage ERT (figure 1C). A similar pattern was observed when comparing survival for final dosages (figure 1D).

Without treatment, patients with classic infantile Pompe disease typically do not achieve the ability to walk. Therefore, we also studied whether treated patients who survived after age 18 months reached this motor milestone. Of the 86 patients with a follow-up beyond 18 months of age, 44 (51%) learned to walk at a median age of 16·0 months (IQR 14·0–19·0, range 11–39 months). The proportion of patients who learned to walk was higher in the high dosage group than the standard and intermediate dosage groups (figure 2A, table 4). Ten (53%) of 19 patients treated with standard dosage ERT learned to walk, whereas six (67%) of nine patients treated with an intermediate dosage and 14 (93%) of 15 patients treated with a high dosage learned to walk. No statistically significant differences with regard to walking were identified between patients given the standard dosage and the high dosage regimen (HR 1·71 [95% CI 0·74–3·98], p=0·21) or between patients given the standard dosage and the intermediate dosage

regimen (0·97 [0·35–2·07], p=0·96). Of the 30 treated patients for whom less than 18 months of follow-up data were available, two learned to walk (one patient received a variable dosage and one received an increased dosage regimen). Of the 44 patients with a follow-up beyond 18 months who learned to walk follow- up data on walking was available for 36 patients. Of these 36 patients, six (17%) lost ambulation at a median age of 53·5 months (IQR 27·8–121·0; figure 2B, table 4). For eight patients, it was unknown whether they sustained the ability to walk (table 4) and 30 patients maintained the ability to walk until their last follow-up at a mean age of 6·8 years (SD 4·5, range 1·8–20·9). Of the 44 patients who learned to walk, five (50%) of ten patients treated with the standard dosage, four (67%) of six patients treated with intermediate dosage, and 12 (86%) of 14 patients treated with a high dosage maintained the ability to walk throughout follow-up.

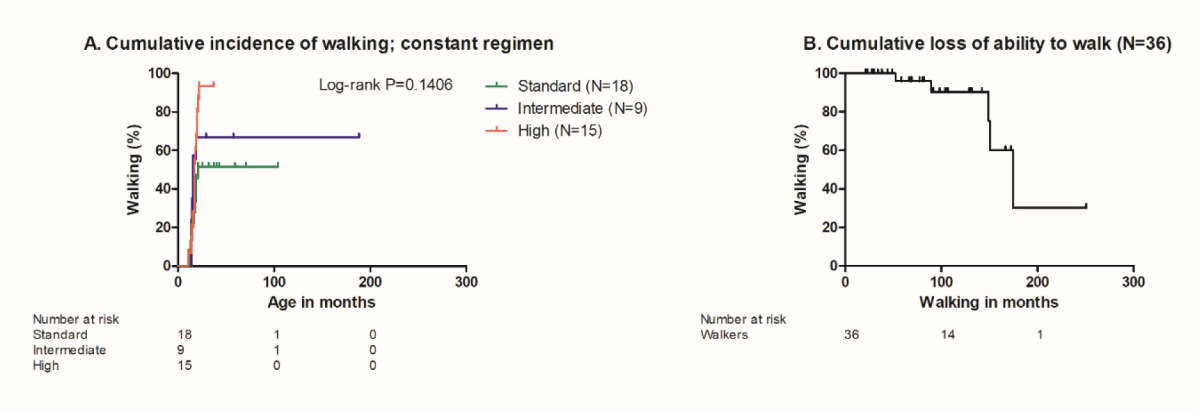

Figure 2: Kaplan-Meier analysis of walking ability and loss of ambulation among patients aged 18 months or older (A) Cumulative incidence of walking among participants given constant regimens who had a follow-up duration of at least 18 months, by age (n=42). (B) Cumulative loss of walking ability by time walking in months (n=36). One patient in the standard treatment group learnt to walk but it was unknown when this patient learnt to walk, thus this patient was not included in this analysis.

	Walking	Not walking
Treatment regimen		
Standard dosage	10/19 (53%)	9/19 (47%)
Intermediate dosage	6/9 (67%)	3/9 (33%)
High dosage	14/15 (93%)	1/15 (7%)
Variable dosage	4/13 (31%)	9/13 (69%)
Decreased dosage	1/4 (25%)	3/4 (75%)
Increased dosage	9/23 (39%)	14/23 (61%)
Unknown	0/3	3/3 (100%)
CRIM-status		
CRIM-positive	29/55 (53%)	26/55 (47%)
CRIM-negative	8/17 (47%)	9/17 (53%)
Unknown	7/14 (50%)	7/14 (50%)
Age when patients started walking		
Overall	16·0 (14·0–19·0)*	NA
Standard dosage	17·0 (13·5–19·0)*	NA
Intermediate dosage	15·5 (14·0–16·8)	NA
High dosage	17·0 (15·8–20·0)	NA
Variable dosage	18·0 (11·8–32·5)	NA
Decreased dosage	21·0 (21·0–21·0)	NA
Increased dosage	16·0 (15·0–18·0)	NA
Age when patient lost walking ability		
Overall	53·5 (27·8–121·0; n=6)	NA

Table 4: Walking ability among patients who reached aged 18 months or older during the study (n=86) Data are n/N (%) or median (IQR). NA=not applicable. *For one patient, the age at which they had learnt to walk was unknown.

Discussion

In this study, we describe the effects of ERT dosage on survival and walking ability in 124 patients with classic infantile Pompe disease, which to the best our knowledge, is the largest real-world international cohort described to date. From 1999 onwards, a plethora of treatment regimens have been implemented in Germany, France, Italy, and the Netherlands. Only 31 (27%) of 116 treated patients received the standard recommended dosage of 20 mg/kg every other week throughout the study period, suggesting that the majority of physicians treating patients with classic infantile Pompe disease are not satisfied with the outcome of their patients on the standard recommended dosage.

The large number of patients in our study has enabled us to demonstrate for the first time that survival is significantly better in patients treated with high dosage ERT (40 mg/kg per week) when compared with survival in patients treated with the standard dosage (20 mg/kg every other week). Additionally, the proportion of patients who learned to walk was highest in the high dosage group, followed by the intermediate dosage group, and the standard dosage group. Despite the marked differences in walking ability between different groups, the difference was not statistically significant, potentially because the number of patients was too small to observe an effect. However, the effect of high ERT dosage on walking ability might be clinically meaningful because the ability to walk is an important developmental milestone and is instrumental to patient independence. These findings are consistent with previous studies that suggested that a higher dosage of alglucosidase alfa might be beneficial in stabilising or improving the clinical condition of patients with classic infantile Pompe disease, with more patients learning to walk and maintaining this ability in the high dosage group.8, 22

The improved clinical effect of higher dosage ERT might be explained by the dose dependent uptake of ERT and concomitant reduction in tissue glycogen content observed in preclinical studies.12,13,29 Uptake of recombinant human α-glucosidase is mediated by the 275 kDa cation-independent mannose-6-phosphate receptor, which is exposed on the cell surface and responsible for endocytosis of exogenous lysosomal enzymes such as α-glucosidase and transport of the enzyme to the lysosomes. The exposure of the receptor on the cell surface varies by tissue type and was found to be significantly lower in skeletal muscle from mice than the heart, diaphragm, and spleen,30 making these tissues easier to target with ERT. Subsequent clinical studies showed dose-dependent clearance of glycogen in skeletal muscle, which was reflected by better clearance of intracellular glycogen in skeletal muscle.3–5,29 It was also shown that the uptake of recombinant human acid α-glucosidase in skeletal muscle was lower than in the heart and that the uptake was dose-dependent. A dosage of alglucosidase alfa 20 mg/kg per

week was found to be sufficient to clear glycogen in cardiac muscle, but insufficient to fully clear glycogen from skeletal muscle cells.13,30,31 Clearance of glycogen in peripheral nerves and vascular endothelium was observed with a dosage of 15 mg/kg per week. However, only after increasing the dosage to 40 mg/kg per week was an effect observed on glycogen storage and pathological changes in skeletal muscle tissue obtained by biopsy.14 Additionally, improvements in muscle morphology were observed with high dosage ERT.3,4

Another factor contributing to the clinical effect of ERT with high dosage alglucosidase alfa is the frequency of enzyme replacement therapy. The intracellular half-life of alglucosidase alfa after uptake is tissue dependent and estimated to be 2–9 days based on preclinical studies.9,10,12 Thus, 14 days between infusions might be too long to achieve optimal clearance of glycogen by intracellular alglucosidase alfa. This could explain why no obvious benefit of 40 mg/kg every other week was observed when compared with 20 mg/kg every other week in the AGLU 01602 trial.5,22 It has been suggested that early treatment with high dosage alglucosidase alfa once per week might benefit patients with classic infantile Pompe disease32 and might result in full correction of biochemical markers of muscle damage during the first years of life, and normal neuromotor development.23 We would like to emphasise that this study assesses the combined effects of dose and frequency of ERT with alglucosidase alfa. Our results, which are consistent with literature, suggest that high dosage ERT given with a high frequency would optimise glycogen clearance from the cells and clinical outcome in patients with classic infantile Pompe disease.

The effect of ERT on Pompe disease is known to be influenced by CRIM status and antibody formation, with a negative CRIM status being associated with an increased likelihood of developing high antibody titres and a poorer clinical outcome on ERT.6,7,19,20 Previously, it has been suggested that higher dosage ERT might overcome the negative effects of CRIM status and antibody formation.22 However, not all problems are overcome with high dosage ERT; for example, some patients experience a clinical decline on treatment after initial response, with many patients developing residual muscle weakness and some patients dying despite treatment. Another important aspect in the treatment of classic infantile Pompe disease is that the current ERT does not cross the blood–brain barrier.
Although we present the largest population of patients with classic infantile Pompe disease studied to date, our study population was not large enough to perform Cox regression with multiple additional coefficients such as CRIM status. CRIM status could be proven or predicted in 83% of 116 patients and

was not evenly distributed across the different treatment groups, with a higher proportion of CRIM-negative patients in the standard dosage treatment group than the high dosage treatment group. Among the patients with a proven or predicted CRIM status, the proportions with CRIM-negative (25%) and CRIM-positive (75%) status were similar to that reported in the literature.5,7,19–22 When CRIM status was included in the Cox regression model, albeit this model being less statistically stable, survival remained significantly improved in the high dosage group when compared with the standard dosage group. This indicates that CRIM status does not explain the difference in survival between treatment groups. Further research is needed for other important outcomes (e.g., ventilator free survival, antibody titre formation) and immunomodulation regimens, when analysing the effect of ERT dosage on outcomes in classic infantile Pompe disease. Our study illustrates that international collaboration within the European Pompe Consortium is instrumental for large cohort studies in Pompe disease and should be pursued.

In conclusion, our study shows that a plethora of treatment regimens are given to patients with classic infantile Pompe disease. From our real-world data, we conclude that survival in patients with classic infantile Pompe disease is significantly improved and that a higher proportion of patients learn to walk when treated with a dosage of 40 mg/kg per week, compared with the current standard dosage of 20 mg/kg every other week. Based on these results, we suggest that the currently registered dosage should be reconsidered.

Contributors

IAMD was involved in data curation, formal analysis, data collection, study design, project administration, data validation and verification, data presentation, and drafting, reviewing, and editing the manuscript. HHH was involved in formal analysis, study design, project administration, supervision, data presentation, and drafting, reviewing, and editing the manuscript. MEK conceptualised the study, and was involved in data curation, data collection, study design, project administration, resource management, and writing, reviewing, and editing the manuscript. DR was involved in formal analysis, and writing, reviewing, and editing the manuscript. AH, TM, FL, and MT conceptualised the study and were involved in data collection, resource management, writing, reviewing, and editing the manuscript. BC and AB were involved in data collection and resource management. RP and GP conceptualised the study and were involved in data collection and resource management. NAMEvdB conceptualised the study and was involved in writing, reviewing, and editing the manuscript. ATvdP conceptualised the study and was involved in formal analysis, study design, project administration, resource management, supervision and leadership, data presentation, and drafting, reviewing, and editing the manuscript. JMPvdH conceptualised the study and was involved in formal analysis, study design, project administration, data validation and verification, resource management, supervision and leadership, data presentation, and drafting, reviewing, and editing the manuscript. The corresponding author takes full responsibility for the data, has full access to all data, and has the right to publish all data.

Declaration of interests

HHH reports advisory board fees, speaker fees, and a clinical trial agreement from BioMarin International to his institution, outside the submitted work. FL reports travel expenses from Sanofi-Genzyme. MT reports personal fees from Sanofi Genzyme, outside the submitted work; and travel expenses from Sanofi-Genzyme. RP reports personal fees from Sanofi-Genzyme and personal fees from BioMarin, outside the submitted work. NAMEvdB received funding for research, clinical trials, and advisory fees from Sanofi-Genzyme and Amicus Therapeutics under agreements between these companies and Erasmus MC University Medical Center. ATvdP received funding for research, clinical trials, and advisory fees from Sanofi-Genzyme, Amicus Therapeutics, Biomarin, Ultragenix, Sarepta, Audentes, and Spark Therapeutics working on enzyme replacement therapy or next-generation therapies in the field of Pompe disease, other lysosomal storage diseases or neuromuscular disorders, under agreements with Erasmus MC University Medical Center and the relevant industry. JMPvdH received funding for research, clinical trials, and advisory fees from Sanofi-Genzyme, Amicus

Therapeutics, Biomarin, Sarepta, and Chiesi working on enzyme replacement therapy or next-generation therapies in the field of Pompe disease, other lysosomal storage diseases or neuromuscular disorders, under agreements with Erasmus MC University Medical Center and the relevant industry. All other authors declare no competing interests.

Data sharing

De-identified data will be shared upon request to the corresponding author from any qualified investigator for the sole purpose of replicating procedures and results presented in the Article, in agreement with EU legislation on the general data protection regulation.

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