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ORIGINAL RESEARCH ARTICLE

Can Alpha-Glucosidase Activity in Plasma or Leukocytes Serve as a Biomarker for Future Gene Therapy in Classic Infantile Pompe Disease?

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Abstract

We studied alpha-glucosidase activity in plasma and leukocytes after an infusion of 40 mg/kg of recombinant alglucosidase alpha in patients with classic infantile Pompe disease to assess the pharmacokinetics and identify potential surrogate efficacy markers of gene therapy in patients on enzyme replacement therapy. Samples were collected by pharmacokinetic curves ($n = 5$) and random samples ($n = 21$ patients). Alpha-glucosidase activity was measured in plasma (substrate 4-methylumbelliferyl-α-D-glucopyranoside, MU) and leukocytes (substrate glycogen, Gn, and MU). Plasma peak concentration occurred at the end of the infusion, reaching concentrations > 5000 and > 100,000 times higher than the control and untreated patient levels, with a median half-life of 3.1 h (1.3–4.2 h). In leukocytes, plasma peak concentration occurred 24 h after the start of enzyme replacement therapy; plasma peak concentration did not exceed the control level (0.7 [Gn] and 0.9 [MU] times higher than controls). The estimated half-life was 2–4 days. Seven days after enzyme replacement therapy, median enzyme activity was 1.3 times higher than the control levels in plasma and within the control range in leukocytes; after 14 days, median values in plasma and leukocytes were below the control level. These findings suggest alpha-glucosidase activity in plasma and leukocytes may serve as an efficacy marker for gene therapy studies in patients with classic infantile Pompe disease receiving enzyme replacement therapy. Similar studies with next-generation enzyme replacement therapy are advised.

Daan Lambregts and Ina Barzel have contributed equally as second authors.

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Graphical Abstract

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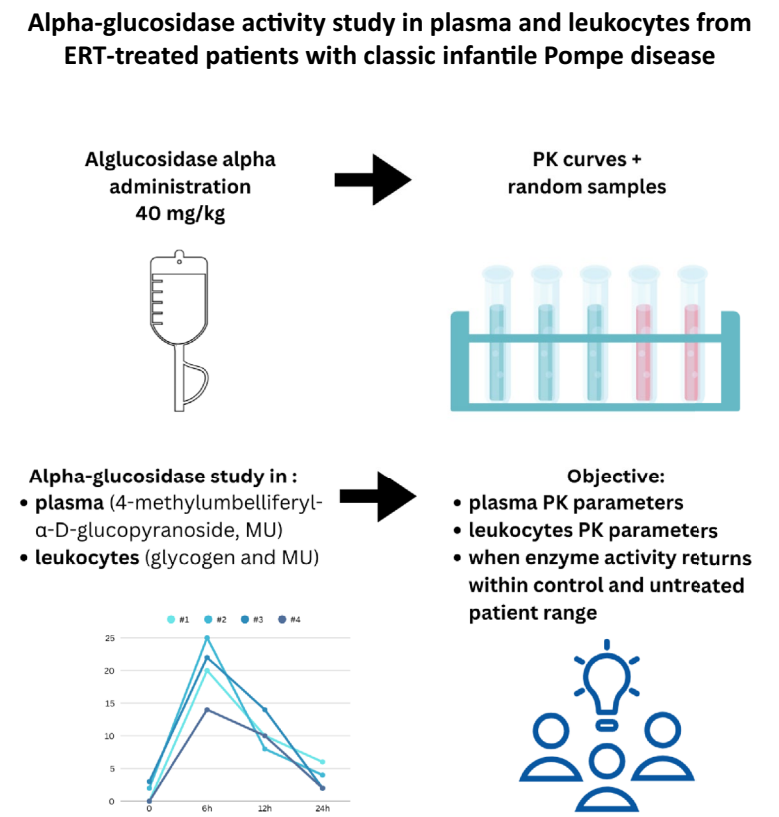
PEER-REVIEWED FEATURE

Can alpha-glucosidase activity in plasma or leukocytes serve as a biomarker for future gene therapy in classic infantile Pompe disease? M.C. Faraguna, D. Lambregts, I. Barzel, E.H. Jacobs, M. Hoogeveen-Westerveld, N.A.M.E van der Beek, P.W.W.M. Pijnappel, S. Gasperini, T. Preijers, J.M.P. van den Hout, A.T. van der Ploeg



Abbreviations: ERT= Enzyme Replacement Therapy; PK= pharmacokinetic

Key Summary Points

In plasma, a high peak concentration of alpha-glucosidase was observed at the end of the infusion and the median half-life was 3.1 h.

In leukocytes, the peak concentration of alpha-glucosidase occurred 24 h after the start of enzyme replacement therapy and did not exceed the control range; the estimated half-life was 2–4 days.

Fourteen days from enzyme replacement therapy, enzyme activities in plasma and leukocytes decreased to a level that any detectable surplus of enzyme activity derived from gene therapy could be detected and distinguished from enzyme replacement therapy.

1 Introduction

Pompe disease (glycogen storage disease type II, OMIM: #232300) is an autosomal, recessive, lysosomal storage disorder caused by deficiency of the enzyme acid alpha-glucosidase (GAA). It is a multi-systemic disease, with a continuum of clinical phenotypes, determined by the amount of residual enzyme activity. Patients with classic infantile Pompe disease express virtually no alpha-glucosidase activity and show the most severe manifestations of the disease, defined by symptom onset before 6 months of age, the presence of a hypertrophic cardiomyopathy, and two severe disease-causing variants in *GAA* [1, 2], as found on the Pompe disease variant database (www.pompevariantdatabase.nl); it usually leads to death by the age of 12 months if left untreated [3].

The introduction of enzyme replacement therapy (ERT) with recombinant human alglucosidase alpha (rhGAA), approved by the European Commission and the US Food and Drug Administration in 2006, has significantly improved the prospects of patients, allowing several to reach adulthood. Long-term follow-up of children with classic infantile Pompe disease reaching adolescence and young adulthood has revealed a previously unknown phenotype, characterized by distal muscle weakness [4, 5], velopharyngeal insufficiency [6], and central nervous system involvement with progressive white matter abnormalities and a gradual decline in cognition and academic performance with age [7–10].

Therefore, the journey towards optimizing therapy is ongoing. Recently, second-generation ERTs (avalglucosidase alfa and cipaglucosidase alfa with miglustat) have been approved, while gene therapies, both with an "in vivo" and "ex vivo" approach, are being developed [11–13]. These advancements aim to address the unmet needs and residual multi-systemic involvement of children treated with ERT.

In the context of developing gene therapy, classic infantile Pompe disease presents a unique challenge compared with other lysosomal storage diseases, as ERT is life saving. Consequently, ERT must be continued during the conditioning period and after gene therapy infusion. The optimal timing for safe discontinuation of ERT after gene therapy is unknown; therefore, it is essential to establish how and when to differentiate the effects of gene therapy from those of ERT. To address this, we investigated the real-life pharmacokinetics of rhGAA following an ERT infusion to (1) determine the plasma half-life of the currently used dosage of 40 mg/kg; (2) study the uptake of rhGAA in leukocytes and determine the half-life in leukocytes; and (3) determine when enzymatic activity returned below controls and within the untreated patient level after an ERT infusion, in order to identify a possible timepoint to evaluate endogenous alpha-glucosidase production after gene therapy.

2 Methods

2.1 Study Design and Participants

Patients with classic infantile Pompe disease were eligible for the study, defined by the onset of symptoms of muscle weakness within 6 months after birth, the presence of hypertrophic cardiomyopathy, confirmed deficiency of alpha-glucosidase in leukocytes and/or fibroblasts, and two very severe mutations in the *GAA* gene (www.pompevariantdatabase.nl). Patients with Pompe disease followed at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center in Rotterdam, the Netherlands are enrolled in the prospective protocol MEC-2007-103-NL, approved by the Medical Ethical Committee of Erasmus Medical Center.

2.2 Pharmacokinetic Curves

Sampling was performed before, during, and after the infusion of alglucosidase alpha at a prescribed dose of 40 mg/kg [14, 15] to assess pharmacokinetic (PK) curves. The prescribed dose was calculated at 40 mg/kg every 3 months; changes in body weight and rounding to whole vials of 50 mg in order not to waste the drug could lead to a variation in the actual dose of ERT administered per kilogram at the time of the PK curve, as reported in Table 1. Blood was taken at fixed timepoints: before the start of ERT ($t = 0$), at the 30-mg/kg dose infusion, at the end of the infusion (total amount of 40 mg/kg infused), at + 15, 30, 60, 120 min, + 4, 8, 16, 24, and 48 h, and 5±1, 7 days from the end

of infusion, depending on the day of the week. Additional timepoints were +3, 9, 12, and 14 days on the basis of the ERT regimen and week day.

During the PK study, blood samples were either drawn from the central catheter, if present, with proper flushing of the line, or from a peripheral vein. The time of the start and end of infusion as well as of blood collection were noted.

During the PK curves, the samples of the first 16 h were stored in the fridge at + 4 °C and then processed. Clinical data including sex, *GAA* mutations, cross-reactive immunological material status, antibody titer, dose of ERT, and duration of infusion were collected.

2.3 Random Sampling

The random samples were collected when the patients visited the hospital for a scheduled appointment and the date and time of the last infusion were noted; in case the patient received an infusion on the same day, the sample was taken before the start of ERT. Data were collected until 28 February, 2025.

2.4 Alpha-Glucosidase Activity Measurement

Alpha-glucosidase activity was measured in leukocytes both with the natural (glycogen, Gn) and artificial (4-methylumbelliferyl-α-D-glucopyranoside, MU) substrate essentially as described before [2, 16] and expressed in nmol/h/mg of protein. Alpha-glucosidase activity in plasma was measured by incubating 10 µL of plasma with 1.47 µM of MU and 3 µM of acarbose (final concentrations) in a volume of 30 µL for 17 h at 37 °C. The activity value was corrected for the percentage of quenching, which was determined by measuring the fluorescence signal after 17 h at 37 °C of either 10 µL of bovine serum albumin (0.2% *w*/*v*) or 10 µL of plasma in a volume of 30 µL with 20 µM of MU (final concentration). The corrected activity was expressed in nmol/17h/mL of plasma.

2.5 Antibody Titers

Samples for the determination of the antibody titer were collected at the time of the PK curve, and were always collected before the start of ERT. A standardized antibody analysis was performed by an enzyme-linked immunosorbent assay [17]. A high antibody titer was defined as ≥1:31250.

2.6 Statistics

Descriptive statistics were used to summarize the demographic and clinical characteristics of the study population. To determine the PK parameters in plasma and leukocytes, a non-compartmental analysis was performed to calculate the peak concentration (C_{max}), time to peak concentration, half-life, and area under the curve extrapolated to infinity using R package "PKNCA version 0.11.0" [18]. To visualize trends in the data, a locally estimated scatterplot smoothing (LOESS) curve was fitted using the LOESS function in R with default parameters.

3 Results

In five patients, the PK analysis was performed in both full plasma and leukocytes after ERT with rhGAA at the dose of 40 mg/kg. Patient characteristics are reported in Table 1. In the PK curve group, four patients received a weekly regimen, while patient 1 initially received a biweekly regimen. This patient was subsequently switched to a weekly regimen after the PK sampling. Therefore, although its PK data reflect a biweekly regimen, its random samples were collected under a weekly regimen. Random sampling of all other patients was performed following weekly infusions.

3.1 Pharmacokinetics of Alpha-Glucosidase in Plasma

Figure 1 shows alpha-glucosidase activity in plasma measured before, during, and after the infusion of rhGAA in five patients (Fig. 1A). For plasma activity measurement, the MU substrate was used.

The C_{max} was observed at the end of the infusion. The median duration of the infusion was 5.1 h (range 4.8–9.0 h). The median C_{max} was 830,405 nmol/17h/mL (range 664,216–1,120,717 nmol/17h/mL) and was > 5000 times higher than the upper level of enzyme activity of normal subjects (range 60–150 nmol/17h/mL) and > 100,000 times higher than the upper limit of the untreated patient range (4–8 nmol/17h/mL). The median half-life and area under the curve were 3.1 h (range 1.3–4.2 h) and 274,627 nmol/mL (range 174,806–394,514 nmol/mL) respectively. To analyze how long the alpha-glucosidase activity remained detectable in plasma after a 40-mg/kg enzyme infusion, we included alpha-glucosidase activity measurements of 21 patients with classic infantile Pompe disease collected when visiting the hospital (n = 182, "random samples"), including data from PK curves; date and time of sampling and of the last infusion were noted.

Enzyme activity was first observed to return to below the lower limit of normal subjects on day 7. On day 7, the median plasma value was 199 nmol/mL/17h (range 47–3770 nmol/mL/17h, n = 35). Twenty-five of 35 samples (71%)

Table 1 Characteristics of patients in whom a PK curve was performed

PT	Sex	Mutations *GAA* gene (NM_001079803.3)	CRIM	Age at PK curve (years)	Antibody titer at PK curve	Duration of ERT infusion (min)	Actual dose of alpha-glucosidase at PK curve (mg/kg)[a]	Immunomodulation, age	Leukocyte count at PK curve (cells/μL)
1	F	c.2104C>T/c.2104C>T	+	8.4	1:6250	306	38	–	6800
2	M	c.2481+102_2646+31del/c.1597T>C	+	6.6	1:156250	290	39	Primary (RTX, MTX, Ig)	11,600
3	F	c.525delT/c.525delT	-	10.4	1:1250	292	31	Secondary, 2.9 years (RTX, MTX, Ig, BTZ); 8.5 years (RTX, MTX, Ig, BTZ; long-term RTX)	5900
4	F	c.634G>T/c.1051del	-	3.3	1:1250	329	40	Primary (MTX); secondary, 1.6 years (RTX, MTX, Ig, BTZ; long-term RTX)	12,800
5	F	c.655G>A/c.655G>A	+	2.5	1:156250	543	40	-	6400

BTZ bortezomib, *CRIM* cross-reactive immunological material, *ERT* enzyme replacement therapy, *F* female, *GAA* acid alpha-glucosidase, *Ig* immunoglobulins, *M* male, *MTX* methotrexate, *PK* pharmacokinetic, *PT* patient, *rhGAA* recombinant human alglucosidase alpha, *RTX* rituximab

[a]The prescribed dose was calculated at 40 mg/kg every 3 months. Changes in body weight and rounding to whole vials of 50 mg in order not to waste the drug could lead to a variation in the actual dose of ERT administered per kilogram at the time of the PK curve. Immunomodulation was administered before starting ERT ("Primary") and/or in the presence of high sustained antibody titers ("Secondary"). Age at immunomodulation cycle is indicated

presented enzyme activity above the upper limit of controls (control range 60–150 nmol/mL/17h). From day 9, no enzyme activity above the control level was detected (n = 11). From day 11, the median value in plasma was 45 nmol/mL/17h (range 38–72 nmol/mL/17h, $n = 7$), below the lower limit of controls (60 nmol/mL/17h, control range 60–150 nmol/mL/17h). On day 14, three samples were available with a median value of 45 nmol/mL/17h (range 40–45 nmol/mL/17h). No enzyme activity below the upper limit of the untreated patient range was detected, even in a patient who did not receive ERT for 20 days (Fig. 1B). This patient presented enzyme activity just above the lower limit of control.

3.2 Pharmacokinetics of Alpha-Glucosidase in Leukocytes

Figure 2 shows the alpha-glucosidase activity in leukocytes measured in the same patients and at the same timepoints as in Fig. 1. Alpha-glucosidase activities were measured both with the natural (Gn, upper panel) and artificial (MU, lower panel) substrate.

The median C_{max} in leukocytes with the Gn assay was 179 nmol/h/mg of protein (range 145–683 nmol/h/mg of protein) and was within the range of controls, i.e., 0.7 times the upper limit of normal (control range 40–250), and was 17.9 times higher than the upper limit of leukocyte activity in patients (patient range 0–10 nmol/h/mg of protein).

The median C_{max} in leukocytes with the MU assay was 24.6 nmol/h/mg of protein (range 16–71 nmol/h/mg of protein) and was within the range of controls, i.e., 0.9 times the upper limit of normal (control range 6.7–27) and was 3.6 times higher than the upper limit of leukocyte activity in patients (patient range 4.9–6.7 nmol/h/mg of protein).

In both assays, the peak was observed after 24 h from the start of ERT (Fig. 2, left panel). The estimated half-life of alpha-glucosidase in leukocytes varied between 2 and 4 days.

To analyze how long the alpha-glucosidase activity remained detectable in leukocytes after a 40-mg/kg enzyme infusion, we included data of 21 patients with classic infantile Pompe disease from random sampling (n = 187 samples for the Gn assay, n = 186 samples for the MU assay), as done for plasma. The enzyme was first observed to return below the threshold of normal subjects on days 6–7 (Gn and MU), and to an untreated patient range at day 14 (Gn) and 7 (MU) (Fig. 2, right panel). On day 7, the median alpha-glucosidase

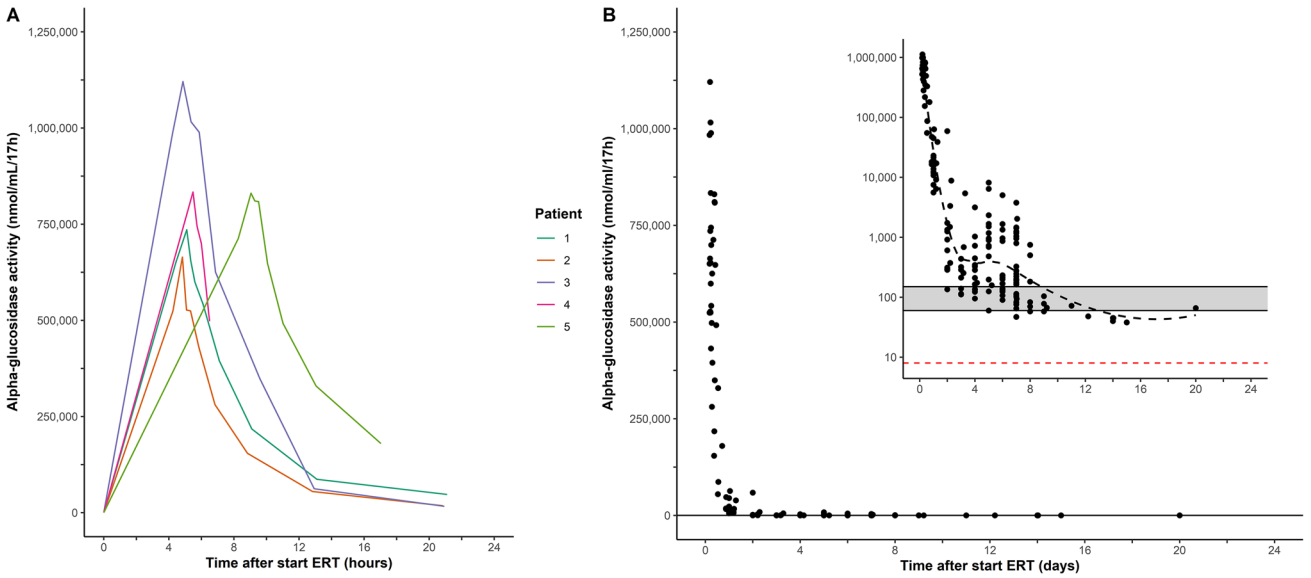


Fig. 1 **A** Alpha-glucosidase activity measured in plasma after a recombinant human alglucosidase alpha infusion in five patients. The pharmacokinetic curve of patient 4 was interrupted after 1 h from the end of enzyme replacement therapy (ERT) because of a catheter dysfunction. **B** Data of plasmatic alpha-glucosidase activity combined from pharmacokinetic curves ($n = 5$) and random sampling ($n = 16$), for a total of 182 samples. No single patient is overrepresented at any specific timepoint, except during the first 24 h, when data are available from the five pharmacokinetic curve patients. From day 11 after the start of ERT onwards, seven measurements from four patients are included. Black line: upper limit of normal alpha-glucosidase activity. The zoom in shows the same data with a logarithm scale on the y-axis, to focus on measurable enzyme activity after 7 days from ERT. Black lines and the gray rectangle represent the control range. Red dotted line: upper limit of the untreated patient level. Black dotted line: non-parametric regression line estimated with "LOESS" function in R

in leukocytes was 80.0 (Gn, range 25.1–137, $n = 37$) and 11.0 (MU, range 3.3–20.9, $n = 37$) nmol/h/mg of protein. No samples were above the upper limit of control. From day 11 onwards, the median value in leukocytes was 12.0 nmol/h/mg of protein (GN, range 0.1–26.3 nmol/h/mg of protein, $n = 7$) and 4.9 nmol/h/mg of protein (MU, range 3–8.1 nmol/h/mg of protein, $n = 7$), below the lower limit of controls (40 nmol/h/mg of protein for Gn and 6.7 nmol/h/mg of protein for MU). On day 14, three samples were available with median values of 12 (Gn, 7.8–15.3) and 4.85 (MU, 2.7–4.8) nmol/h/mg of protein. Two samples collected at day 15 and 20 from ERT revealed enzyme activity within the patient range both in the Gn and MU assays.

4 Discussion

In this study, we analyzed alpha-glucosidase activity in plasma and leukocytes following an rhGAA infusion at the prescribed dose of 40 mg/kg. We focused on the C_{max}, the half-life, and the remaining enzymatic activity before the next infusion, to evaluate when enzyme activity dropped below the control level and within the untreated patient range after ERT, in order to identify a potential window to evaluate endogenous alpha-glucosidase production after gene therapy in future clinical studies.

First, we performed five PK curves and analyzed the plasmatic levels of alpha-glucosidase before, during, and after the infusion. At the end of the infusion, we observed a very high plasmatic peak, with the median C_{max} exceeding the upper level of normal subjects by > 5000 times and of patients by > 100,000 times. The C_{max} obtained in this study was not directly comparable to that reported by the Summary of Product Characteristics, which reports protein concentration (in microgram/milliliter) [19] instead of alpha-glucosidase activity. Elimination from circulation was rapid, with a median half-life of 3.1 h, similar to the mean half-life of 2.75 h reported in the Summary of Product Characteristics for children receiving 40 mg/kg of alpha-glucosidase [19]. Of the five PK curves, two were performed in the presence of high antibody titers: patient 2 showed the lowest C_{max}, while in patient 5 C_{max} occurred later because of a slower infusion. To fully understand the effect of antibodies on enzyme activity, it should be measured in plasma with the addition of artificial beads of protein A, to quantify the amount of antibody-bound enzyme [15, 17].

Subsequently, it was investigated when enzymatic activity after ERT returned below the control level and within the untreated patient range. Enzyme activity declined over

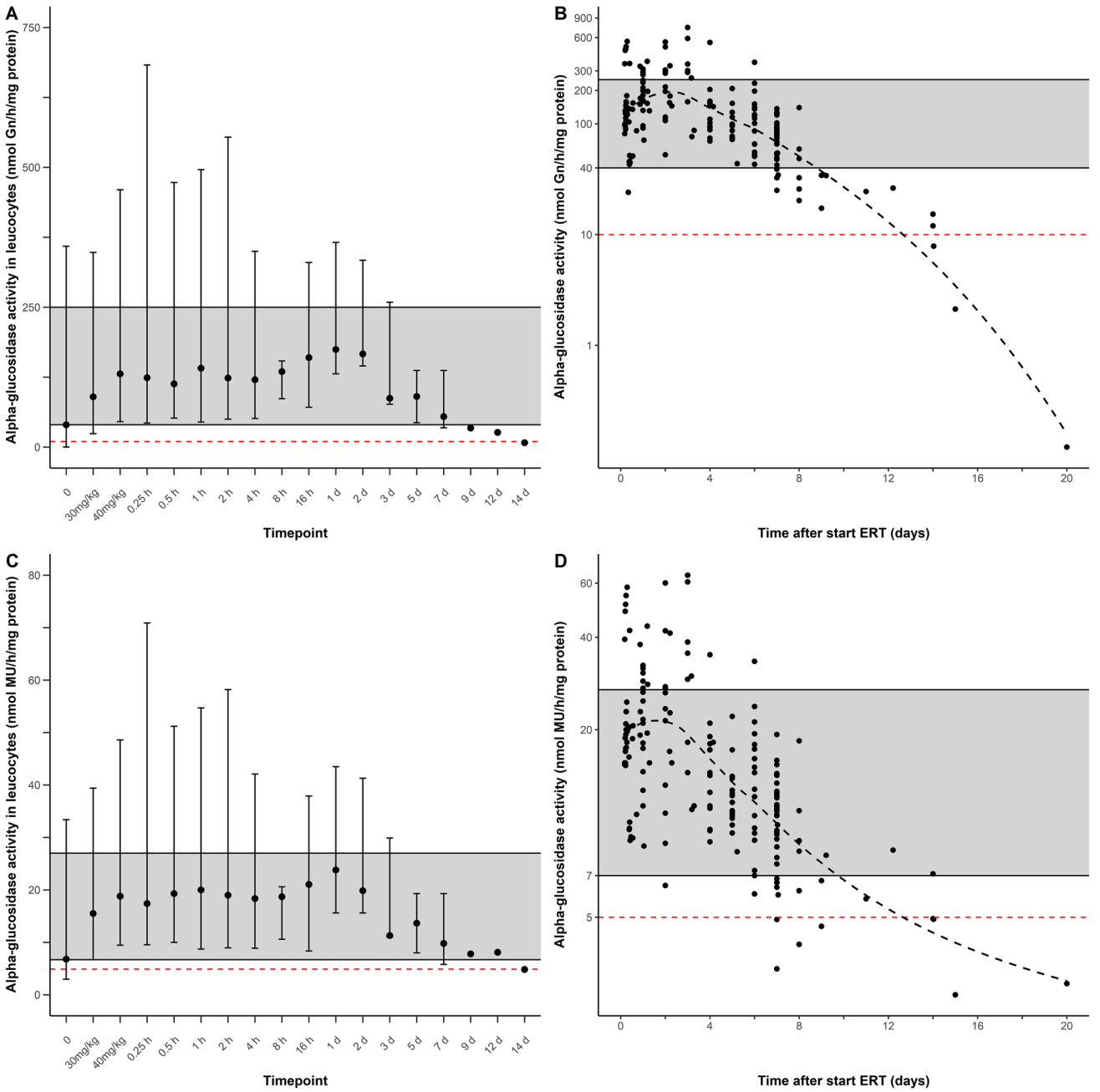


Fig. 2 Alpha-glucosidase activity measured in leukocytes with the glycogen (**A**) and 4-methylumbelliferyl-α-D-glucopyranoside (**C**) substrate during five pharmacokinetic curves. The median value is represented by the black dot and the black bars represent the minimum and maximum value measured. In (**B**) and (**D**), pharmacokinetic data were combined with random sampling. The y-axis shows the logarithmic scale to focus on the remaining enzyme activity before the next infusion. Black lines and the gray rectangle represent the control range. Red dotted line: upper limit of the patient level. The dotted line depicts the locally estimated scatterplot smoothing (LOESS) line. *d* days, *ERT* enzyme replacement therapy, *h* hours

time to stabilize at a plateau between the control and patient level; no enzyme activity was detected within the untreated patient level, even in a single observation of a child with classic infantile Pompe disease who did not receive ERT for 20 days. The Summary of Product Characteristics of alglucosidase alpha does not report PK data of patients receiving weekly infusions. However, in patients with late-onset Pompe disease treated with 20 mg/kg every other week, C_{max} remained stable at weeks 0, 12, and 52, not revealing an accumulation over time [20]. Pharmacokinetics may differ with weekly ERT administration, which is the standard for patients with classic infantile Pompe disease. Persistence of

alpha-glucosidase activity, above the patient range, was also described 48 h after administration of neo-rhGAA in patients with late-onset Pompe disease treated with 5, 10, or 20 mg/kg of avalglucosidase alpha every other week [21].

The source of the remaining enzyme activity between the control and untreated patient range is still not clear. Earlier studies in mice have shown that, after ERT administration, alpha-glucosidase activity increases by > 100 times in the liver and spleen in comparison to wild-type [22–24]. Furthermore, a persistence of high enzymatic activity in the liver and spleen was observed up to 15 days post-ERT administration in mice (6.5 and 26.5% of endogenous activity in the liver and spleen, in comparison to 1.1% in the muscle) [22]. Enzyme activity has not yet been studied in the liver and spleen after ERT infusion in humans. However, we can speculate that the persistence of alpha-glucosidase activity described in this study, mostly between the normal and disease ranges, could be related to the large quantities of ERT taken up by liver, spleen, and the reticulo-endothelial system [25, 26]. These organs may act as reservoirs, slowly releasing the enzyme back into the circulation. Currently, the exact biological path of the remaining enzyme remains unclear. Lysosomal exocytosis is increasingly recognized as a vital cellular process and occurs in all cell types; the remaining enzyme activity we measured in plasma might be excreted from the lysosomes [27–29], but this requires further studies.

We then analyzed alpha-glucosidase activity in leukocytes after an rhGAA infusion. Leukocytes display mannose-6-phospate receptors on the cellular membrane, which is the gate through which rhGAA enters the cells [30]. Previous studies have demonstrated that untreated patients exhibit periodic acid-Schiff (PAS)-positive lymphocyte vacuoles, with PAS staining used to highlight glycogen accumulation [31]. Within a few weeks of starting ERT, the percentage of PAS positive vacuoles and PAS score greatly decreased in all patients [31], indicating that ERT entered the cell. Given their accessibility, leukocytes have been used as a model to study the cellular uptake and clearance of alpha-glucosidase, as well as the intra-lysosomal half-life. Similar studies have been performed for other lysosomal storage diseases [32, 33].

After an rhGAA infusion, the peak of alpha-glucosidase activity observed in leukocytes was quite low. In particular, the median C_{max} was 18 (Gn)/4 (MU) times higher than the upper limit of the untreated patient range and did not exceed control levels, i.e., 0.7 (Gn) and 0.9 times (MU) the upper limit of normal values. The peak concentration after ERT was quite modest, especially when compared with the extremely high peaks in plasma, which were > 5000 times higher than the upper level controls and > 100,000 times higher than untreated patient levels. Yet, previous studies have shown that this level is sufficient to reduce glycogen accumulation in lymphocytes within a few weeks of starting ERT [31]. This suggests that even a modest increase in enzyme activity can have therapeutic effects.

However, muscle tissue differs fundamentally. Unlike leukocytes, which are directly exposed to circulating alpha-glucosidase, muscle cells are shielded by many physiological barriers, such as the endothelial barrier and the endomysium [34]. This means that while leukocyte enzyme activity may provide insight into enzyme uptake dynamics, it does not fully reflect the challenges of enzyme delivery to muscle tissue. If leukocytes, despite their direct exposure to circulating enzyme, only show a modest increase in activity, this raises concerns about whether a sufficient amount of enzyme reaches muscle cells.

The modest C_{max} might be explained by the low content of mannose-6-phosphate of rhGAA. To overcome this, second-generation ERT such as avalglucosidase alpha was developed with an increased number of mannose-6-phosphate groups to optimize cellular uptake [21]. We therefore speculate that the low C_{max} in leukocytes, despite their direct exposure to very high plasma concentrations of rhGAA, may be due to the molecule's insufficient phosphorylation.

The peak concentration in leukocytes was observed about 24 h from the start of the infusion, later in comparison to plasma C_{max} (at the end of the infusion). Furthermore, the half-life was 2–4 days, similar to early findings in mice after the injection of bovine testes-derived alpha-glucosidase [22] and fibroblasts of patients with Pompe disease cultured with bovine testes alpha-glucosidase [35], which is also in net contrast to the short half-life in plasma. Two patients received long-term rituximab, which depletes CD20-positive cells. As alpha-glucosidase activity is measured in leukocytes (neutrophils, lymphocytes, monocytes) and overall counts remained within the normal range, we do not expect this to have altered enzyme activity.

This study aimed to identify a potential timepoint to assess a surrogate efficacy marker of gene therapy, such as alpha-glucosidase activity in plasma and leukocytes. In patients with classic infantile Pompe disease, ERT must be continued before, during, and after gene therapy, making it essential to determine a specific moment when enzyme activity can be evaluated without the influence of ERT. In the presence of enzyme activity derived from gene therapy, such findings must be integrated with age-appropriate clinical parameters to consider safe discontinuation of ERT. We found that, in plasma, at day 7, 70% of samples expressed levels above the control range, at day 9, all enzyme activities were within or below the control level, and from day 11, 70% of samples were below the lower limit of control. Enzyme activity in plasma did not return to the untreated patient range, even after 20 days from ERT in one patient. In leukocytes, at day 7, 100% of samples were within or below control levels (Gn and MU); in the glycogen assay,

from day 9, all samples were below the lower limit of control and from day 14 within the patient range. In the MU assay, from day 11, 70% of samples were below the lower limit of control and from day 14 onwards within the patient range. Enzyme activity measurement in leukocytes with the glycogen assay was the most consistent, with all samples being below the control range from day 9. These findings suggest alpha-glucosidase activity in plasma and leukocytes may serve as a surrogate marker for future gene therapy studies, even for patients with classic infantile Pompe disease on ERT. In fact, after 14 days from ERT, enzyme activities in plasma and leukocytes decreased to a level that any detectable surplus of enzyme activity derived from gene therapy could be detected and distinguished from ERT. For example, in the mouse model, lentiviral gene therapy with an insulin-like growth factor 2 tag added to a codon-optimized version of GAA (LV-*IGF2.GAAco*) led to a > 120 times increase of alpha-glucosidase activity in leukocytes in comparison to the knock-out mouse and a > 30 times increase in comparison to wild-type [11]. Future studies will assess whether similar findings of this study hold for second-generation ERTs.

A limitation of this study is represented by the limited amount of data available after 9 days from ERT; given the variability in enzyme activity at earlier timepoints, more samples are necessary to confirm the trend we observed. In classic infantile Pompe disease, the standard of care of our center consists of weekly infusions; therefore, it is not frequently possible to obtain such data; so said, we will continue collecting data.

5 Conclusions

We reported data on the pharmacokinetics of alglucosidase alpha after an infusion of 40 mg/kg in classic infantile Pompe disease. Analyzing the trajectory of enzyme activity after ERT, we found that alpha-glucosidase measurement in leukocytes with the glycogen assay was the most consistent. After 14 days from ERT, enzyme activities in plasma and leukocytes decreased to a level that any detectable surplus of enzyme activity derived from gene therapy could be detected and distinguished from ERT. Future studies will assess whether similar findings hold for second-generation ERTs.

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Declarations

Conflicts of Interest/Competing Interests Ans T. van der Ploeg and Johanna M.P. van den Hout received funding for research, clinical trials, and/or as an advisor from various industries working on ERT or next-generation therapies in the field of Pompe disease under agreements with Erasmus MC University Medical Center and the relevant industry. Pim W.W.M. Pijnappel is a co-founder and unpaid Chief Scientific Officer of LentiCure, a company developing gene therapy for lysosomal disorders including Pompe disease. Nadine A.M.E van der Beek has received consulting fees for advisory boards or speaker honoraria from Sanofi, Amicus Therapeutics, Shionogi, and Bayer under agreements with Erasmus MC University Medical Center and the relevant industry. Ed H. Jacobs and Marianne Hoogeveen-Westerveld received funding for clinical trial and contract work from Spark Therapeutics and Amicus Therapeutics. Martha C. Faraguna, Daan Lambregts, Ina Barzel, Serena Gasperini, and Tim Preijers have no conflicts of interest that are directly relevant to the content of this article.

Ethics Approval Patients with Pompe disease followed at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center in Rotterdam, the Netherlands are enrolled in the prospective protocol MEC-2007-103-NL, approved by the Medical Ethical Committee of Erasmus Medical Center.

Consent to Participate Patients or their parents/legal guardians provided written consent to participate in the study.

Consent for Publication Patients or their parents/legal guardians consented to publication of this study.

Availability of Data and Material All original data are present in the article. Further enquiries can be addressed to the corresponding author.

Code Availability Enquiries concerning the code can be addressed to the corresponding author.

Authors' Contributions MCF participated in planning the project and design of experiments, collected data, analyzed and interpreted data. and wrote the draft of the manuscript. ATP and JMPH conceived the project, designed experiments, interpreted data, participated in discussions, and edited several versions of this manuscript. IB and TP contributed to the PK analysis, results interpretation, improvement of figure quality, and reviewed several versions of the paper. DL, SG, PWWMP, and NAME participated in data interpretation and reviewed several drafts of the manuscript. EHJ and MHW supervised enzyme activity measurement and the enzyme-linked immunosorbent assay, analyzed and interpreted data, and contributed to the manuscript preparation.

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