ORIGINAL ARTICLE

Effects of higher and more frequent dosing of alglucosidase alfa and immunomodulation on long-term clinical outcome of classic infantile Pompe patients

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Abstract

The aim of this study was to compare the long-term outcome of classic infantile Pompe patients treated with 20 mg/kg alglucosidase alfa every other week (eow) to those treated with 40 mg/kg/week, and to study the additional effect of immunomodulation. Six patients received 20 mg/kg eow and twelve 40 mg/kg/week. Five patients were cross-reactive immunologic material (CRIM)-negative, two in the 20 mg, three in the 40 mg group. We compared (ventilator-free) survival, motor outcome, infusion associated reactions (IARs), and antibody formation. From 2012 on patients >2 months in the 40 mg group also received immunomodulation with rituximab, methotrexate, and intravenous immunoglobulin (IVIG) in an enzyme replacement therapy (ERT)-naïve setting. Survival was 66% in the 20 mg group and 92% in the 40 mg group. Ventilator-free survival was 50% and 92%. Both CRIM-negative patients in the 20 mg group died, whereas all three are alive in the 40 mg group. In the 20 mg group, 67% learned to walk compared with 92% in the 40 mg group. At the age

Abbreviations: AIMS, Alberta Infant Motor Scale; BSID-II, Bayley Scales of Infant Development II; CRIM, cross-reactive immunological material; ELISA, enzyme-linked immuno sorbent assay; ERT, enzyme replacement therapy; eow, every other week; GAA, acid-α-glucosidase; IAR, infusion associated reaction; IVIG, intravenous immunoglobulin; LSDs, lysosomal storage disorders; LVMI, left ventricular mass index; rhGAA, recombinant human GAA.

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of 3 years, 33% and 92% were able to walk. Peak antibody titers ranged from 1:1250 to 1:31 250 in the 20 mg group and from 1:250 to 1:800 000 in the 40 mg group. Five patients of the 40 mg group of whom two CRIM-negative also received immunomodulation. B-cell recovery was observed between 5.7 and 7.9 months after the last dose of rituximab. After B-cell recovery titers of patients with and without immunomodulation were similar (ranges 1:6 250-1:800 000 and 1:250-1:781 250). This study shows that classic infantile patients treated with 40 mg/kg/week from the start to end have a better (ventilator-free) survival and motor outcome. Immunomodulation did not prevent antibody formation in our study.

KEYWORDS

anti-rhGAA antibody titer, cross-reactive immunologic material (CRIM), enzyme replacement therapy (ERT), glycogen storage disease type II, immunomodulation, Pompe disease

1 | INTRODUCTION

Intravenous enzyme replacement therapy (ERT) with recombinant human enzymes is the treatment of choice for several lysosomal storage disorders (LSDs).[1] Among them is Pompe disease (glycogen storage disease type II, OMIM: #232300), an autosomal recessive LSD caused by deficiency of the enzyme acid-α-glucosidase (GAA).[2,3] The disease spectrum is broad. The most severe classic infantile form presents shortly after birth with a hypertrophic cardiomyopathy and generalized muscle weakness. Without therapy patients die within the first year of life.[4,5] Major motor milestones like walking are not achieved, GAA deficiency is profound (<1%) and the *GAA* variants are very severe.[3,6,7] One-third of the patients are cross-reactive immunologic material (CRIM) negative and do not produce any alpha-glucosidase protein. The alpha-glucosidase protein produced by CRIM-positive patients is not adequately transported to the lysosomes and inactive. The registered dose of recombinant human GAA (rhGAA, alglucosidase alfa) for all patients is 20 mg/kg every other week (eow).[2,3] This dose is mainly based on the results of the initial pivotal clinical trial (AGLU 01602) in which patients received either 20 or 40 mg/kg eow.[8] This trial in 18 classic infantile patients lasted 52 weeks and all survived beyond the first year of life. There was no clear difference between the outcomes of the 20 or 40 mg/kg eow group. A longer follow-up study showed that there is still room for improvement as nearly 50% of patients did not survive ventilator-free beyond the age of 3 years and 56% did not learn to walk.[8,9] More recent longer follow-up studies report similar results.[10-12]

Clinical and preclinical studies have shown that skeletal muscles are difficult to target and that uptake is dose dependent.[13-17] Other factors that have shown to be of influence on outcome are CRIM status and antibody formation.[18-20] CRIM-negative patients have shown to perform poorly and tend to produce high anti-rhGAA antibody titers. The outcome of CRIM-positive patients seems more variable. In these patients, high sustained titers have also been reported to have a negative effect.[18,19,21-23]

To improve clinical outcomes, we have investigated the effect of a higher dose of 40 mg/kg/week. This dose was applied in our very first clinical trial with recombinant human alpha-glucosidase produced in milk from transgenic rabbits in 1999. This study showed that enzyme activity levels in skeletal muscle were higher with a dose of 40 mg/kg/week than with 15 or 20 mg/kg/week.[15] From 2009 onwards, all our newly diagnosed patients received 40 mg/kg/week from the start. In 2016, we published our preliminary results in eight CRIM-positive patients comparing 20 mg/kg eow with 40 mg/kg/week.[20] Here, we present our longer follow-up data in a larger cohort of 18 patients also including CRIM-negative patients. From 2012 onwards, we further investigated whether immunomodulation, using a protocol published by Messinger et al,[24,25] aimed to prevent the production of anti-rhGAA antibodies, in combination with a higher dose had added benefit.

2 | METHODS

2.1 | Patients and treatment

In this prospective standardized follow-up study, all Dutch patients diagnosed with classic infantile Pompe disease who were treated with rhGAA (Alglucosidase

alfa, Myozyme) from the start were included. The Pompe Center at Erasmus MC is the single reference center for ERT of Pompe patients in the Netherlands. Classic infantile Pompe disease was defined as (a) symptoms of muscle weakness within 6 months after birth, (b) the presence of hypertrophic cardiomyopathy, (c) deficiency of endogenous α-glucosidase in fibroblasts (<1% of the normal values), and (d) pathogenic variants in the GAA gene.[3] ERT was dosed at 20 mg/kg eow between 2003 and 2009. From 2009 onwards, all newly diagnosed patients received 40 mg/kg/week. Dosing of all patients receiving 20 mg/kg eow was increased to 40 mg/kg/week somewhere between 2009 and 2014, due to clinical deterioration. In 2012, immunomodulation with rituximab (RTX), methotrexate (MTX) and intravenous immunoglobulins (IVIG) in an ERT-naïve setting was initiated in newly diagnosed patients older than 2 months of age at the time of diagnosis (see for details[25]). Antibiotic prophylaxis was given to prevent (respiratory) infections. Data until December 31st 2016 were analyzed.

2.2 | Clinical efficacy

Standardized assessments were performed at baseline and every 3 months thereafter.[16,20] Study protocols were approved by the Institutional Review Board and written informed consent was obtained from the parents and/or patients. Clinical outcome was assessed by (ventilator-free) survival. Cardiac dimensions were measured by conventional echocardiography; the left ventricular mass index (LVMI) was calculated by the Devereux formula and indexed by body surface area. An LVMI of > +2 SD of age-related peers was considered abnormal.[26] Motor function was assessed by Alberta Infant Motor Scale (AIMS) and Bayley Scales of Infant Development II (BSID-II), and motor milestones were recorded.[27,28] Results were compared to age-related healthy peers. Infusion associated reactions (IARs) were noted when they occurred. The severity of each IAR was indexed by clinical judgment as mild/moderate or severe.

2.3 | Antibody detection

Anti-rhGAA titers were assessed at the start of ERT and thereafter at 3, 6, 9, 12, 18, 24 months and from then on yearly. When receiving immunomodulation, titers were also determined at 1 and 2 months after the start.[25] Standardized antibody analysis was performed by an enzyme-linked immunosorbent assay (ELISA) as previously described.[19,22,29] Antibody titers were measured in 5-fold serial dilutions. Samples were measured in duplicate. Assays were performed at least twice. The background of the ELISA method was

determined to be 1:250 by omitting the coating of the plates with rhGAA as described previously.[22]

3 | RESULTS

3.1 | Patients

Eighteen patients were included, nine (50%) were male. Table 1 compares the patient groups treated with 20 mg/kg eow (n = 6) and 40 mg/kg/week (n = 12). Age at start of treatment was younger in the 20 mg group and ranged from 0.1 to 3.6 months (median 1.5 months) compared to 0.3 to 5.9 months (median 3.6 months) in the 40 mg group. Two patients in the 20 mg group were CRIM-negative and three in the 40 mg group. Age at the last assessment ranged from 0.6 to 12.6 years (median 9.6 years) and from 3.0 to 8.3 years (median 4.4 years) for both groups. Four patients treated with 20 mg/kg eow had their dose increased to 40 mg/kg/week at ages ranging from 1.5 to 9.4 years either because of serious respiratory infections, becoming ventilator dependent or motor decline. Figure S1 shows the treatment regimen per patient.

3.2 | Effects of higher dosing on clinical outcomes

At the study end, survival was 66% in the 20 mg group and 92% in the 40 mg group (P = .25). (Figure 1). Three patients died (aged 0.6, 3.1, and 4.4 years) due to respiratory failure; both CRIM-negative patients from the 20 mg group and one CRIM-positive patient from the 40 mg group. Ventilator-free survival was 50% for the 20 mg group and 92% for the 40 mg group (P = .08).

The ability to walk was achieved by four patients (67%) in the 20 mg group and by 11 (92%) in the 40 mg group. By the age of 3 years, two (33%) in the 20 mg group and 11 (92%) patients in the 40 mg group maintained the ability to walk (P = .02). At the end of the study, respectively, one (17%) and 10 (83%) were persistent walkers.

Figure 1 and Table 1 show the AIMS and BSID-II scores per treatment group. Although the ranges in AIMS and BSID-II scores were similar, only patients from the 40 mg group (n = 6 of whom two were CRIM-negative) reached the maximum AIMS score of 58. The median BSID-II age-equivalent score at 36 months was higher for the 40 mg group, 30 months, compared to 20 months for the 20 mg group. Only 50% of patients in the 20 mg group compared to 92% of patients in the 40 mg group could be adequately tested with the BSID-II at 36 months. The test was not performed in the others either because they had died (n = 3) or were ventilator-dependent (n = 1).

TABLE 1 Patient characteristics and outcome comparing 20 mg/kg eow with 40 mg/kg/week

	20 mg/kg eow	40 mg/kg/week
Patient characteristics		
Number of patients	6	12
Males (%)	4 (67%)	5 (42%)
Median age at start in months (range)	1.5 (0.1-3.6)	3.6 (0.3-5.9)
Number of CRIM-negative (%)	2 (33%)	3 (25%)
ERT dose increase	4 (67%)[a]	N.A.
Age at dose increase in years (median, range)	4.1 (1.5-9.4)	N.A.
Outcome		
Median age at last assessment in years (range)	9.6 (0.6–12.6)	4.4 (3.0-8.3)
Survival (%)	66%	92%
Survival of CRIM-negative patients (%)	0%	100%
Ventilator-free survival (%)	50%	92%
Median LVMI z-score at start (range)	6.15 (2.4-8.6)	7.1 (3.0-13.7)
Number of patients with LVMI normalization during follow-up (%)	5 (83%)	11 (92%)
Median ERT duration at LVMI normalization in years (range)	0.5 (0.25-1.71)	0.5 (0.25-1.4)
Best motor milestone (%)		
MMF	1 (17%)	0
Sitting	1 (17%)	1 (8%)
Walking	4 (67%)	11 (92%)
Number of patients walking independently at age 3 years (%)	2 (33%)	11 (92%)
Last motor milestone (number, %)		
MMF	1 (17%)	0
Sitting	4 (67%)	2 (17%)
Walking	1 (17%)	10 (83%)
AIMS at 12 months (median, range)	37 (20-45)[b]	39 (20-50)
AIMS at 18 months (median, range)	54 (25-57)[b]	57 (34-58)
BSID-II AE score at 24 months (median, range)	17 (10.4-21)[c]	18 (14-25)
BSID-II AE score at 36 months (median, range)	20 (20-32)[c]	30 (19-33)[d]
Median peak antibody titer (range)	1:6250 (1250-31 250)	1:156250 (250-800 000)
Tube feeding at start (number, %)		
NGT	6 (100%)	9 (75%)
PEG	0	0
Oral	0	3 (25%)
Tube feeding at study end (number, %)		
NGT	1 (17%)	1 (8.5%)
PEG	2 (33%)	1 (8.5%)
Oral	3 (50%)	10 (83%)
Number of patients with IARs (total number of IARs)	5 (64 IARS, 4 severe)	8 (134 IARS, 11 severe)

Abbreviations: AIMS, Alberta Infant Motor Scale; BSID-II AE; Bayley Scales of Infant Development II age-equivalent score; CRIM, cross-reactive immunological material; ERT, enzyme replacement therapy; IARs, infusion associated reactions; LVMI, left ventricular mass index; MMF, minimal motor function; NGT, nasogastric tube; PEG, percutaneous endoscopic gastrostomy.

[a]Dose increase was only applied in the surviving four patients from the 20 mg group.

[b]AIMS at 12 and 18 months was performed in the five surviving patients.

[c]BSID-II was performed in three patients, the two patients requiring invasive ventilation were not tested due to illness.

[d]BSID-II at 36 months was performed in the surviving 11 patients, two patients were 34 months of age at time of testing.
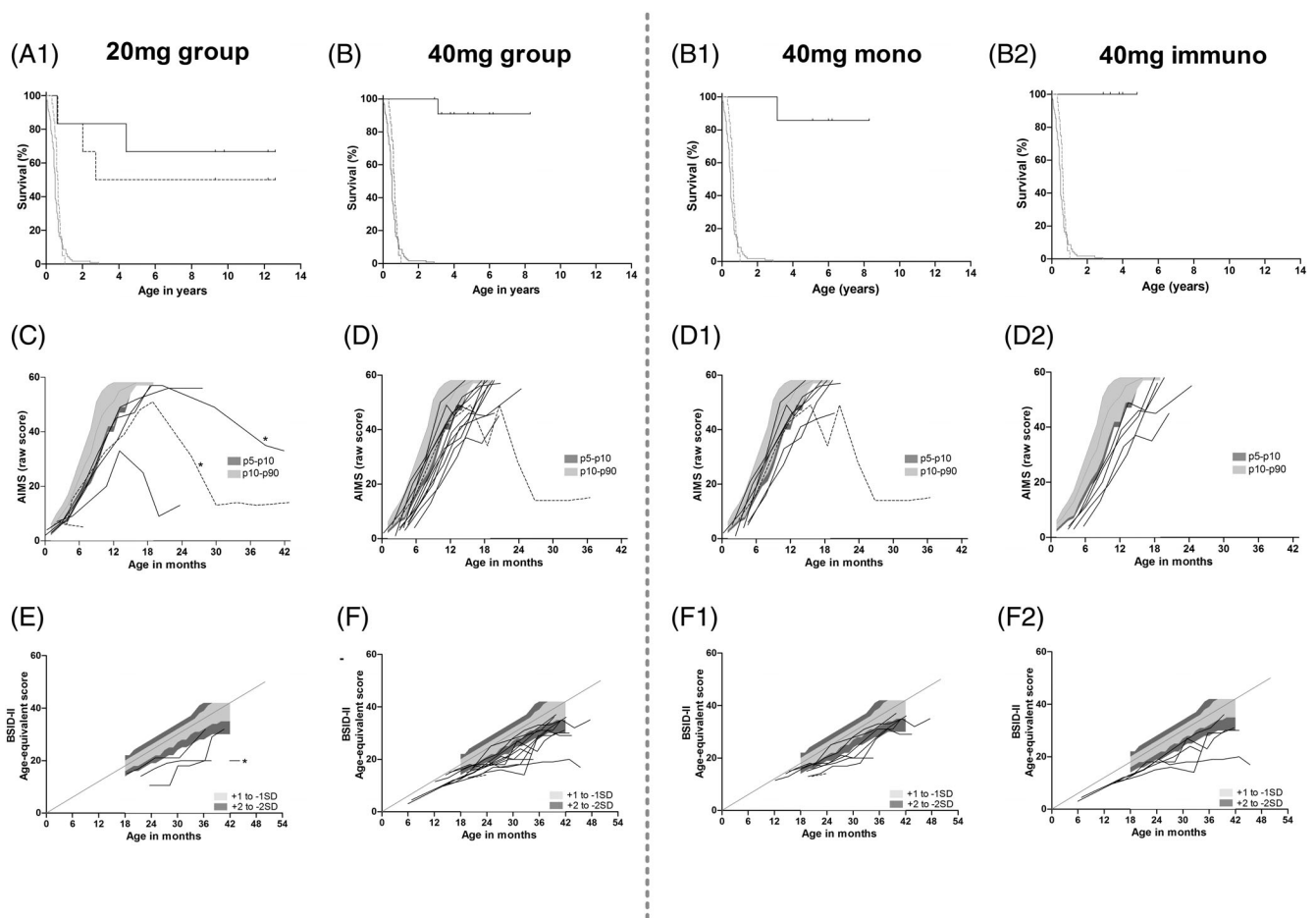
FIGURE 1 Survival and motor outcome. A, Survival in patients receiving 20 mg/kg ERT eow and B, 40 mg/kg/week. Gray lines represent the historical cohorts. C, AIMS score in patients receiving 20 mg/kg eow and D, 40 mg/kg/week. E, BSID-II score in patients receiving 20 mg/kg/eow and F, 40 mg/kg/week. In the right part of the panel data, patients treated with 40 mg/kg/week were subdivided in outcomes for patients receiving 40 mg/kg/week ERT monotherapy and patients receiving 40 mg/kg/week ERT with immunomodulation. B1-B2, Survival; D1-D2, AIMS score; F1-F2, BSID-II score. Dashed lines in C-F represent the deceased patients. The asterisks mark the patients who required invasive ventilation. AIMS, Alberta Infant Motor Scale; BSID-II, Bayley Scales of Infant Development II; ERT, enzyme replacement therapy

The cardiac response, effect on LVMI, did not show considerable differences between the two groups (Figure S2). The LVMI did not normalize in one patient who died after 3 months of treatment (20 mg group). Two patients in 40 mg group had a severe dilated cardiomyopathy at start, which responded well to treatment. In one of those patients, LVMI was still slightly elevated at the last assessment +2.1 SD (+13.7 SD at start).

3.3 | Additional effect of immunomodulation in patients receiving 40 mg/kg/week

Five of 12 patients in the 40 mg group received additional primary immunomodulation (immunomodulation in an ERT-naïve setting) with rituximab, methotrexate, and IVIG. This protocol was introduced in 2012 in patients

older than 2 months of age to prevent antibody formation and to further improve outcomes. Start ERT ranged from 0.1 to 3.6 months in the 20 mg group and from 0.3 to 5.9 months in the 40 mg group. We first compared the clinical outcome of patients treated with 40 mg/kg/week monotherapy (40 mg mono group, n = 7, CRIM-negative 1) and those treated with 40 mg/kg/week plus immunomodulation (40 mg immuno group, n = 5, CRIM-negative 2, Table 2). The 40 mg immuno group was slightly older (range 3.1-5.8 months) compared to the 40 mg mono group (range 0.3-4.8 months) at start.

At the end of the study, (ventilator-free) survival was 86% (six of seven) in the 40 mg mono group and 100% (five of five) in the 40 mg immuno group (Table 2). The patient who died was CRIM-positive. The ability to walk was achieved by six of seven patients (86%) in the 40 mg mono group and by five (100%) in the 40 mg immuno group. All patients were still able to walk at the age of 3 years. At the

TABLE 2 Patient characteristics and outcome comparing 40 mg/kg/week monotherapy to 40 mg/kg/week with immunomodulation

	40 mg/kg/week monotherapy	40 mg/kg/week immunomodulation
Patient characteristics		
Number of patients	7	5
Males (%)	3 (43%)	2 (40%)
Median age at start in months (range)	3.1 (0.3-4.8)	4.3 (3.1-5.8)
Number of CRIM-negative (%)	1 (14%)	2 (40%)
Outcome		
Age at last assessment in years (median, range)	6.0 (3.1-8.3)	3.8 (3.0-4.8)
Survival (%)	86%	100%
Ventilator-free survival (%)	86%	100%
Median LVMI z-score at start (range)	7.0 (3.0-9.6)	7.8 (4.0-13.7)
Number of patients with LVMI normalization during follow-up (%)	7 (100%)	4 (80%)
Median ERT duration at LVMI normalization in years (range)	0.9 (0.25-0.75)	0.5 (0.25-1.4)
Best motor milestone (number, %)		
MMF	0	0
Sitting	1 (14%)	0
Walking	6 (86%)	5 (100%)
Number of patients walking independently at age 3 years (%)	6 (86%)	5 (100%)
Last motor milestone (number, %)		
MMF	0	0
Sitting	2 (29%)	0
Walking	5 (71%)	5 (100%)
AIMS at 12 months (median, range)	41.5 (27-50)	36 (20-49)
AIMS at 18 months (median, range)	58 (34–58)	56 (35-58)
BSID-II AE score at 24 months (median, range)	18 (14-25)	18 (15-19)
BSID-II AE score at 36 months (median, range)	31 (30-33)	23 (19-31)
Peak antibody titer (median, range)	1:156 250 (250-781 250)	1:468750 (6250-800 000)
Antibody titer at last assessment (median, range)	1:31 250 (250-781 250)	1:93750 (250-781 250)
Tube feeding at start (number, %)		
NGT	4 (57%)	5 (100%)
PEG	0	0
Oral	3 (43%)	0
Tube feeding at study end (number, %)		
NGT	1 (14%)	0
PEG	1 (14%)	0
Oral	5 (71%)	5 (100%)
IARs in number of patients (total number of IARs)	6 (110 IARS, 6 severe)	2 (24 IARs, 5 severe)

Abbreviations: AIMS, Alberta infant motor scale; BSID-II AE; Bayley Scales of Infant Development II age-equivalent score; CRIM, cross-reactive immunological material; ERT, enzyme replacement therapy; IARs, infusion associated reactions; LVMI, left ventricular mass index; MMF, minimal motor function NGT, nasogastric tube; PEG, Percutaneous endoscopic gastrostomy.
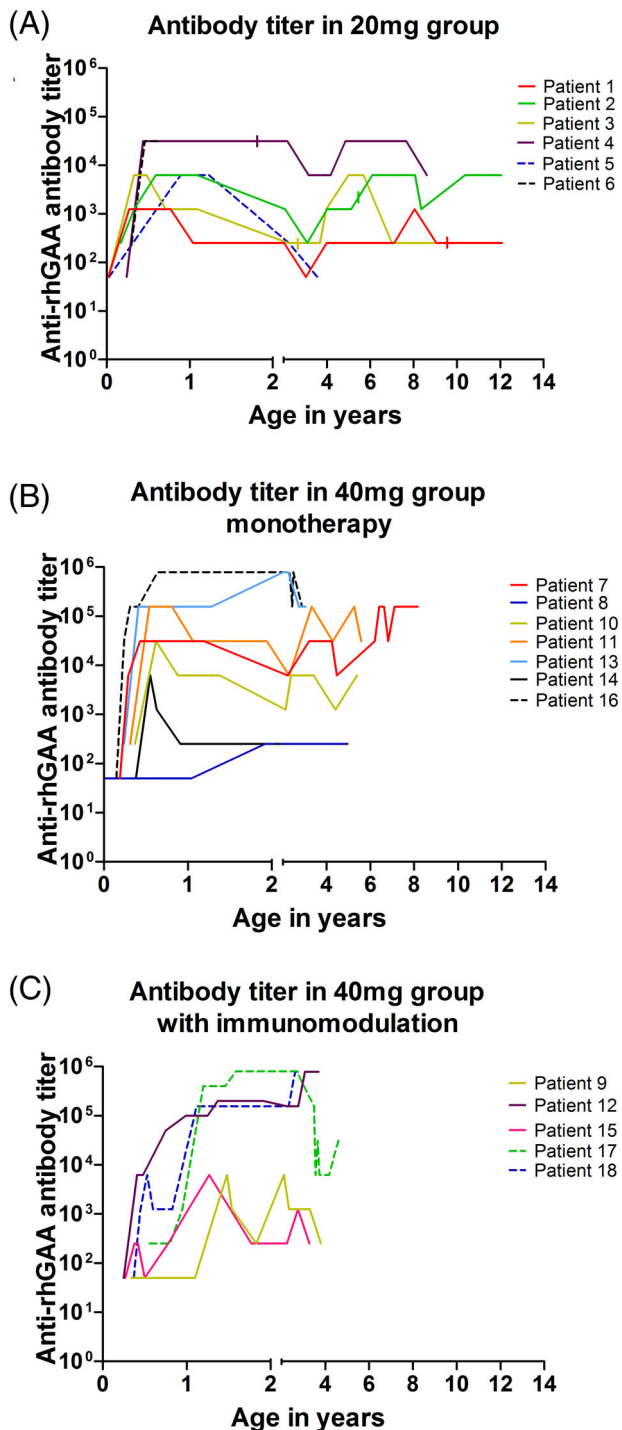
FIGURE 2 Anti-rhGAA antibody titers in patients receiving 20 mg/kg ERT eow compared to 40 mg/kg/week monotherapy and 40 mg/kg/week and immunomodulation. Each line represents an individual patient. Dashed lines indicate the CRIM-negative patients per group. A, Anti-rhGAA antibody titers in patients receiving 20 mg/kg eow; B, Anti-rhGAA antibody titers in patients receiving 40 mg/kg/week monotherapy. C, Anti-rhGAA antibody titers in patients receiving 40 mg/kg/week and immunomodulation in an ERT-naïve setting. CRIM, cross-reactive immunological material; ERT, enzyme replacement therapy; rhGAA, Recombinant human acid-α-glucosidase

end of the study, five (71%) in the 40 mg mono group and five (100%) in the 40 mg immuno group were persistent walkers, including all three CRIM-negative patients. The age range of patients at the last assessment was 3.1 to 8.3 (median 6.0) years for 40 mg mono group and 3.0 to 4.8 (median 3.8) years for the 40 mg immuno group. Figure 1 and Table 2 show the AIMS and BSID-II scores per treatment group. The ranges in AIMS and BSID-II scores were similar. Also, response on LVMI was similar.

3.4 | Anti-rhGAA antibody titers and IARs

When comparing antibody titers between the 20 mg and the combined 40 mg groups peak titers ranged from 1:1250 to 1:31 250 in the 20 mg group (median 1:6250) and from 1:250 to 1:800 000 (median 1:156 250) in the 40 mg group. Two patients in 20 mg group developed high (sustained) titers of 1:31 500 (one was CRIM-negative), and seven in the 40 mg group (see for details below). Figure 2 shows anti-rhGAA antibody titers during follow-up per patient.

Immunomodulation resulted in B-cell depletion in all patients receiving immunomodulation. B-cell recovery was observed between 5.7 and 7.9 months after the last dose of rituximab.[25]

Comparison between the 40 mg mono and the 40 mg immuno groups showed peak titers ranging from 1:250 to 1:78 1250 (median 1:156 250) for the 40 mg mono group and titers ranging from 1:6250 to 1:800 000 (median 1:468 750) for 40 mg immuno group. All CRIM-negative patients in 40 mg group developed high sustained titers irrespective of whether they received immunomodulation; four of the nine CRIM-positive patients developed high sustained titers, one had received primary immunomodulation.

IARs were observed in all groups. Five patients (83%) in the 20 mg group and eight (67%) in the 40 mg group experienced IARs. Comparison between 40 mg mono and 40 mg immuno groups revealed that six (86%, CRIM-positive and CRIM-negative) and two (40%, CRIM-negative) patients experienced IARs, respectively. In all, but two patients (both CRIM-negative), IARs were treated successfully and had not reoccurred for at least 12 months.

4 | DISCUSSION

The primary aim of this study was to investigate the role of higher ERT dosing on clinical outcomes. ERT has generally improved short-term survival of patients, but with longer follow-up it has become evident that there are still

considerable unmet clinical needs as 50% of the reported patients do not survive ventilator-free at the age of 3 years and even lower numbers obtain or maintain the ability to walk.[8,9] The current study shows that classic infantile patients receiving an ERT dose of 40 mg/kg/week from start tend to have a better survival and ventilator-free survival (92% for both outcomes) than patients who started on 20 mg/kg eow (66% and 50%). They also tend to have a better motor outcome: 92% achieved the ability to walk and 83% are still able to walk at study end (median age 4.4 years, range 3.0-8.3 years), compared to 67% and 17%, respectively, in the 20 mg group (median age 9.6 years, range 0.6-12.6 years). At the age of 3 years, 92% and 33% were able to walk. The latter effect was significant. Most notable was that all three CRIM-negative patients in the 40 mg group were alive at study end and able to walk compared to none in 20 mg group. Five patients of the 40 mg group (of whom two of the three CRIM-negative) also received immunomodulation in an ERT-naïve setting. Immunomodulation did not prevent antibody formation in our study. All patients receiving immunomodulation were alive and persistent walkers at the end of the study.

Studies from various countries have reported the long-term outcome of patients with classic infantile Pompe disease: the UK, Germany, and Italy, and the initial pivotal trial (AGLU 01602).[8-12] Most of the patients (n = 102) in these combined studies started ERT on 20 mg/kg eow. The maximum follow-up of patients in these studies ranged from 3 to 13.6 years. The median follow-up duration of the 01602 extension study was the shortest (2.3 years). Reported survival ranged from 56% to 66% and ventilator-free survival was 29% to 50% in the various studies. The ability to walk was achieved in 25% to 44%, and 19% to 39% of patients were persistent walkers at study end. The maximum follow-up in our study was 12.6 years for the 20 mg group and 8.3 years for the 40 mg group. The clinical results of our 20 mg group are similar to the results published in literature (67% for survival, 50% for ventilator-free survival, 67% walkers and 17% persistent walkers). In our study, the surviving four patients of the 20 mg group were all increased in dose to 40 mg/kg/week at ages ranging from 1.5 to 9.4 years because of clinical deterioration. Khan et al recently also reported that a standard dose of 20 mg/kg eow is insufficient to halt progression of disease and that 40 mg/kg/week should be considered in patients with clinical and functional decline.[30] Unfortunately, in our study, dose increase after deterioration could not prevent that only one is a persistent walker.

A potential explanation for the better outcome in the 40 mg group is that a higher dose is required to obtain sufficient uptake by muscle cells and transport of rhGAA to the lysosomes, and a more frequent dose to maintain a sufficient GAA activity level in the lysosomes for glycogen clearance.[13,17] The estimated intracellular half-life of GAA after uptake based on preclinical studies is 3 to 7 days. Fourteen days between infusions may be too long. It should be noted that ERT in other LSDs, such as MPS I, II, IV, and VI are also administered weekly.[1] This could explain why in the AGLU 01602 trial no obvious clinical benefit of 40 mg/kg eow over 20 mg/kg eow was observed.[8,9] It was further noted by the authors of the AGLU 01602 trial that there was an over-representation of CRIM-negative patients receiving 40 mg/kg eow that may have also contributed to the limited effect. A recent study of Spada et al also suggested that weekly higher dosing from start might lead to a better outcome.[31]

Regarding antibodies, we found anti-rhGAA antibody titers in both groups. High sustained antibody titers of ≥1:31 500 developed in four of the nine CRIM-positive and all three CRIM-negative patients in the 40 mg group and in one CRIM-positive patient in the 20 mg group. This might indicate that antibody titers were generally higher in the higher dose group, but it should also be noted that these patients were generally older at start. Earlier, we observed that patients who started ERT within the first 2 months of life seemed to develop lower titers than those who started ERT later.[19,20] Figure S3 shows antibody titers in patients who started ERT before and after 2 months of age and might point in the same direction. Further research is needed. Regarding the inhibitory effect of antibody titers, we previously found that patients with titers <1:31 250 did not show inhibitory effects of anti-rhGAA antibody titers in enzyme uptake experiments and immunoprecipitation[19] and calculated that in a patient with a titer of 1:156 250, with a blood volume of 80 mL/kg and an ERT dose of 20 mg/kg, 54% of the infused enzyme (about 10 mg/kg) could possibly be bound to antibodies.[19,29] Theoretically, with a higher dose more antibody-free rhGAA will be available and the anticipated neutralizing effects of antibodies less severe. This may explain why we observed a good clinical outcome in most of our patients despite high titers and why all our CRIM-negative patients in 40 mg group are currently alive and persistent walkers, where other studies rarely report survival in CRIM-negative patients.

The second aim was to investigate whether immunomodulation could further improve clinical outcomes. In patients receiving immunomodulation in an ERT-naïve setting in combination with a high dose we achieved 100% ventilator-free survival and all are persistent walkers. It should be noted that this group of patients was the youngest of the groups that we compared with the shortest follow-up. Immunomodulation has become a major focus in the treatment of classic infantile Pompe

patients to improve clinical outcomes.[10,24,25,32-34] We applied immunomodulation protocols in an ERT-naïve setting similar to what was reported in literature.[24,25] The effect of our immunomodulation regimen on the antibody titers was limited. There are several reports that were successful in eliminating and/or preventing antibody formation. In Reference [25], we reviewed results and the regimens applied to 24 patients receiving immunomodulation in an ERT-naïve setting reported in the literature. The age range of these patients at the last assessment was 4 to 49 months. At the time of the report, 19 of 24 were alive (survival 79%) and 17 of 24 (71%) survived ventilator free. Thirteen of the 24 patients were older than 18 months at time of report; six of these 13 patients learned to walk (46%); five of these (38%) were persistent walkers. Only two patients were 3 years or older (36 and 49 months). Both patients did not achieve the ability to walk (see Reference [25] for review).

Most of these patients were CRIM-negative and all patients started ERT on 20 mg/kg eow, yet some were receiving a higher dose at study end. This shows that standard dose in combination with immunomodulation not always leads to a good outcome. Kazi et al reported on 19 CRIM negative patients, age at study end was 7.1 to 103.9 months (median 30.1) who received immunomodulation in an ERT naïve setting. Survival in this group was 73% (14 of 19), 36% (7 of 19) was ambulatory and 36% (7 of 19) were ventilator free at last assessment. In this study, the dose varied.[36]

We hypothesize that in our study a higher more frequent ERT dose is the main reason for the good clinical outcome. Immunomodulation may have contributed to the clinical stability of our patients, but it did not prevent antibody formation in our study. It should be noted however that the higher dose cannot overcome all limitations. Many patients in the 40 mg group still have residual muscle weakness and some also lose motor milestones. Thus, the road to a cure for classic infantile patients does not end here. This also includes the implementation of successful immunomodulatory regimens to prevent or reduce high antibody titers, which we also consider of utmost importance.

It should be noted that our study had several limitations. Although the number of classic infantile patients is relatively large, the absolute number and numbers of subjects in the subgroups are still small. Findings have to be interpreted cautiously. Due to small sample size statistics could only be applied for a limited number of outcome measures.

Finally, we found high sustained antibody titers and IARs in both dose groups, in both CRIM-positive and CRIM-negative patients. The relationship between the antibody titers and IARs is very complex and warrants further investigation.

In conclusion, the present study shows that our classic infantile patients receiving 40 mg/kg/week from start have a better survival, ventilator-free survival and motor outcome than patients receiving 20 mg/kg eow. Most notable, all three CRIM-negative patients in the 40 mg group were alive and able to walk at study end irrespective of whether they received immunomodulation.

CONFLICT OF INTEREST
AvdP, JvdH, and NvdB have provided consulting services for various industries (Sanofi-Genzyme, Amicus Therapeutics, Biomarin, Spark Therapeutics, Sarepta Therapeutics, GSK, Shire, Audentes Therapeutics) in the field of Pompe disease and other lysosomal storage disorders under an agreement between these industries and Erasmus MC, Rotterdam, The Netherlands. The other authors declare that they have no conflict of interest. No funding sources had any influence on the content of this manuscript nor provided editorial support.

AUTHOR CONTRIBUTIONS
Esther Poelman participated in the study design, data analyses and data interpretation, and drafted the manuscript. Jan J. A. van den Dorpel contributed to the analyses, data interpretation, and manuscript revision. Marianne Hoogeveen-Westerveld carried out the biochemical assays, participated in the design of the study. Johanna M. P. van den Hout contributed to the data interpretation and revised the manuscript. Lianne J. van der Giessen contributed in data acquisition and data interpretation and revised the manuscript. Nadine A. M. E. van der Beek contributed to the data interpretation and revised the manuscript. W. W. M. Pijnappe participated in its design and interpretation, and revised the manuscript. Ans T. van der Ploeg conceived the study,

participated in its design and interpretation, and acted as principal investigator. We declare that all authors have read and approved the final version of the manuscript, have fulfilled the criteria for authorship, agree with its contents, and take full responsibility for the manuscript.

ETHICS STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.