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Current Opinion in Immunology

Review

A comprehensive study on the effect of alglucosidase alpha and immunomodulation on survival, motor and cardiac outcome, creatine kinase and antibody titers in classic infantile Pompe disease: the Monza experience

Martha C Faraguna[1,2,*], Viola Crescitelli[1,*], Sonia Bonanomi[1], Gaia A Kullmann[3], Maria L Boffi[6], Roberta Pretese[1], Giulia Risca[4], Lucia M Tedesco[5], Katia Pozzi[1], Marta Serafini[2,7], Johanna MP van den Hout[8], Ans T van der Ploeg[8], Adriana C Balduzzi[1] and Serena Gasperini[1]

We report on 13 classic infantile Pompe patients, including four cross-reactive immunological material negative (31%), treated with alglucosidase alpha (rhGAA) at Fondazione IRCCS San Gerardo, Monza, between 2003 and 2024. Median age at rhGAA initiation was 3.3 months, with nine patients (69%) starting on doses > 20 mg/kg/every other week. With a median follow-up of 6.9 years, the 5- and 10-year survival was 92%. Four patients died, and three became ventilator-dependent. Hypertrophic cardiomyopathy normalized in all patients, though electrocardiogram abnormalities persisted in 36%. Walking was achieved by 10 (77%). Ten patients received immune tolerance induction (77%, five primary, two secondary, three both), and seven received long-term sirolimus. Nine developed anti-rhGAA, of whom five (38%) had high-sustained antibody titers (HSAT). All had elevated creatine kinase at diagnosis; creatine phosphokinase normalized over time in four patients on 40 mg/kg/week since start without HSAT. This study offers real-world insight into managing classic infantile Pompe disease and compares the cohort's outcome to international experiences.

Addresses
[1] Pediatrics, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
[2] School of Medicine and Surgery, University of Milano Bicocca, Monza, Italy
[3] Child Neuropsychiatry, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
[4] Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, School of Medicine and Surgery, University of Milano-Bicocca, 20854 Vedano al Lambro, Italy
[5] Physical Medicine and Rehabilitation Unit, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
[6] Cardiology, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy
[7] Tettamanti Center, Fondazione IRCCS San Gerardo Dei Tintori, Monza, Italy
[8] Center for Lysosomal and Metabolic Diseases, Department of Pediatrics, Erasmus MC University Medical Center, Rotterdam, the Netherlands

Corresponding author: Gasperini, Serena (serena.gasperini@irccs-sangerardo.it)
[*] Contributed equally as first authors.

Current Opinion in Immunology 2026, **99**:102725

This review comes from a themed issue on **Paediatric immunology**

Edited by **Pietro Invernizzi**

For complete overview of the section, please refer to the article collection, "PAEDIATRIC IMMUNOLOGY (2025)"

Available online 22 January 2026

Received: 18 October 2025; Revised: 30 December 2025; Accepted: 2 January 2026

https://doi.org/10.1016/j.coi.2026.102725

Introduction

Pompe disease (OMIM 232300) is caused by the deficiency of the lysosomal enzyme alpha-glucosidase (GAA), which leads to glycogen accumulation. The wide phenotypic variability depends on the residual GAA activity, determined by the severity of mutations in the GAA gene [1,2]. Classic infantile Pompe disease is defined by the onset of signs of the disease in the first six months of life, the presence of hypertrophic cardiomyopathy (HCMP), and a virtually absent GAA activity. It usually leads to death in the first 12 months of life if not treated [3]. Patients with classic infantile Pompe disease can be distinguished into cross-reactive immunological material (CRIM) negative patients, who do not produce any trace of native GAA enzyme, and CRIM positive ones, who produce a small amount of nonfunctional protein [4,5].

While enzyme replacement therapy (ERT) with recombinant human alglucosidase alfa (rhGAA) has

changed the natural course of the disease [6,7], with major benefits on cardiomyopathy and enabling achievement of motor milestones that would otherwise be unattainable, a substantial variability in long-term outcomes remains a major challenge [8–13]. Treatment response varies among patients, partly due to differences in ERT dose and regimen, immune response, and age at treatment initiation. Over the past two decades, a lot has been learnt about the pathophysiology and the multisystemic involvement of the disease, driving important advances in its management and treatment. The licensed dose of rhGAA for the classic infantile form is 20 mg/kg/every other week. Early studies demonstrated that patients treated with 40 mg/kg/week achieved a better outcome than those receiving lower or less frequent doses [14,15], prompting some centers to adopt higher dosing [16]; these findings, particularly regarding overall survival (OS) and walking ability, were later confirmed by larger cohort studies [17,18]. Second, most classic infantile Pompe patients who are treated with ERT develop antidrug antibodies. Historically, high anti-rhGAA antibody titers (defined as ≥1:31250 [19] or ≥1:12800 [20], depending on the type of assay) were attributed to a negative CRIM status [5,21]. To date, it is known that up to 40% of CRIM-positive patients also develop high titers [4,22]. Antidrug antibodies can antagonize the effect of therapy [5,23]. Predicting who will develop high-sustained antibody titers (HSAT) remains challenging. Other reported risk factors include older age at start of therapy, higher doses of ERT [5,19,24–26], as well as specific human leukocyte antigens [27]. Third, age at start of treatment has a positive effect on motor development and survival because the disease is characterized by progressive glycogen accumulation, earlier initiation of ERT is consistently associated with better outcomes [28].

The aim of this study was to perform a comprehensive analysis of the long-term outcome of a cohort of patients with classic infantile Pompe disease, focusing on survival, motor and cardiac outcomes, and comparing these findings to previously published cohorts. In addition, the study examined the antibody response in relation to both primary and secondary immunomodulation strategies, including the long-term use of sirolimus. Finally, the potential of creatine kinase as a biomarker was investigated.

Methods

Study design

An observational retrospective single-center study was performed at the Metabolic Center of the Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy. The study was approved by the Pediatric Italian National Ethical Committee, protocol no. 198. Written consent was obtained from the parents/legal guardians.

Subjects with a diagnosis of classic infantile Pompe disease, defined by the onset of symptoms of muscle weakness by 6 months of age, the presence of HCMP, a severe deficiency of GAA activity, and two severe mutations of the GAA gene (www.pompevariantdatabase.nl), treated with rhGAA were eligible for the study.

Data was collected from 01/01/2003 to 15/11/2024. Patient observation for the purpose of this study ended at data lock (15/11/2024), death, or rhGAA discontinuation (i.e. switch therapy). Partial data of patients have been published in previous studies [10,17].

Cross-reactive immunological material status

CRIM status was determined in fibroblasts or peripheral blood and/or based on information provided in the Pompe variant database [31,32].

Enzyme replacement therapy

ERT regimen was classified as constant or modified according to Ditters et al [17]. The dose was classified as high (40 mg/kg/week), intermediate (20 mg/kg/week or 40 mg/kg/2 weeks), or standard (20 mg/kg/2 weeks).

Immunomodulation protocol

The primary immune tolerance induction (ITI) protocol adopted by our center was the one published by Banugaria and colleagues [29] and consisted of:

-intravenous immunoglobulins (Ig) 400 mg/kg, administered at day −2 of ITI and then at week 4, then monthly until B cell levels and IgG levels had reached total Ig values > 500 mg/dL,

-intravenous rituximab (RTX) 375 mg/sqm/dose or 12.5 mg/kg if body surface area < 0.5, at day −1 and then weekly for 4 weeks,

-subcutaneous or oral methotrexate (MTX) 0.4 mg/kg/dose, administered on the day of ERT and on the two following days for 3 weeks in total.

The primary ITI protocol was tailored case by case and administered in a ERT-naïve setting.

Secondary immunomodulation was administered in case of high-sustained antibody titers in ERT-experienced patients; each patient received an individualized immunomodulation scheme; in most cases, a single dose of rituximab (375 mg/sqm) was prescribed.

In addition, since 2019, on a case-by-case basis, oral sirolimus has been implemented at a dose of 0.5 mg/sqm/day. The dose was adjusted based on serum sirolimus levels (target range 4–12 μg/L, preferably around 7–8), which were measured by liquid chromatography tandem mass spectrometry.

Laboratory testing

Blood samples to assess creatine kinase (CK), Pro–B-Type Natriuretic Peptide (Pro-BNP), and anti-alglucosidase alpha (anti-rhGAA) antibodies were collected before ERT infusion every 3–6 months. Anti-rhGAA antibody titers were determined by enzyme-linked immunosorbent assay by the Sanofi Genzyme/LabCorp laboratory. A high sustained antibody titer (HSAT) was defined as ≥1:12800 in two different measurements with an interval of 6 months [20].

Motor evaluation

The following milestones were analyzed: the ability to sit without support, the ability to walk without support, and the loss of walking.

Cardiac function

Cardiac dimensions were measured by conventional 2D echocardiography M-mode; left ventricular internal cavity dimension in diastole, interventricular septum thickness in diastole, left ventricular posterior wall thickness in diastole, and Ejection Fraction were recorded. The left ventricular mass index (LVMI) was calculated by the Devereux formula and indexed by body surface area (LVMI z-score). An LVMI z-score of > +2 standard deviations (SD) of age-related peers was considered abnormal [30]. Time to heart normalization was defined as the time from the start of ERT to the first detection of LVMI z-score < 2 SD. Electrocardiograms (ECGs) were re-analyzed by a pediatric cardiologist (L.B.) to evaluate electrical abnormalities at the last follow-up.

Statistical analyses

Categorical variables were summarized by counts and percentages, while continuous ones were described by median and ranges/minimum-maximum. Kaplan–Meier estimator, with 95% confidence interval (CI), was applied to describe OS, ventilator-free survival, and walking ability, and the log-rank test was used to determine differences in comparing the standard high dose to the other regimens. Tests were two-sided with a significance level of 0.05. A Wilcoxon rank-sum test was used to compare the time to cardiac normalization between the high dose at ERT initiation and other regimens. A one-sided test was applied at a significance level of 0.05. Statistical analyses were performed using R version 2024.04.1.

Results

Population

Thirteen patients were included in the study (five male, eight female). Two patients were siblings. The median age at diagnosis was 3.1 months (range 0–11.5). Median duration of follow-up was 6.9 years (range 1.9–18.4). Clinical and genetic characteristics of the patients are reported in Table 1. The GAA variants of 11 patients could be found in the Pompe Variant Database [31,32]. Patients 11 and 13, siblings, carried a novel mutation in homozygosity, which leads to the deletion of exon 3, causing premature truncation of the protein in correspondence of amino acid 95. CRIM status was determined in peripheral blood and resulted in a negative. Two additional patients in the cohort were CRIM negative (4/13, 31%).

Survival outcome

Four patients deceased during follow-up at ages of 1.7, 11.5, 13.7, and 18.4 years, including two CRIM-negative. Five- and 10-year OS was 92.3% (95% CI 0.79–1), with a median follow-up of 6.9 years. Three patients became ventilator dependent (23%) at the ages of 0.3, 0.8, and 2.6 years. Ventilator-free survival was 75.2% at 5- and 10-years (95% CI 0.54–1) (Figure 1a,b).

Enzyme replacement therapy

All patients were treated with rhGAA. The median age at the start of therapy was 3.3 months (range 0.3–12.9). Four patients initiated ERT at the standard licensed dose of 20 mg/kg/ every other week, while the remaining nine patients received higher doses (three intermediate, six high) from the start. Six patients were treated with a constant ERT regimen (one intermediate, five high), while in seven patients the dose and/or frequency was modified: it was increased in five cases, while the dose was variable in patients 4 and 8 due to infusion-associated reactions (IARs) (Figure 1c). All patients diagnosed after 2019 (n = 5) were started at the dose of 40 mg/kg/week. Eight of 13 (62%) patients received home ERT after 6–12 months of hospital infusions without adverse events, according to Italian regulations [34]. Two patients were not eligible for home ERT because of regional regulations and received ERT at the local hospital.

Motor outcome

All patients learnt to sit without support, at a median age of 7.9 months (range 5.0–18.4); 10 of 13 patients (77%) learnt to walk (median 12.5 months, range 12.0–20.0), amongst whom all six patients were treated with 40 mg/kg/week from start (p=0.39). One patient subsequently lost the ability to walk at the age of 17.5 years. No differences were observed in the sitting acquisition milestone between the patients who started on 40 mg/kg/week and those who did not (p=1).

Heart outcome

All patients presented HCMP at diagnosis; cardiac assessments of patients 11 and 13 were performed in another hospital and were not available, but were previously published [33]. The median LVMI z-score of the remaining 11 patients at diagnosis was +6 SDs for body surface area (range 3.6–13.4), and the median LVMI was 142.2 g/sqm (range 93.8–237.8). Left

Table 1

Patient characteristics.

PT	Sex	GAA variants	CRIM	Age at diagnosis (months)	Age at start ERT (months)	Alive	Age end of follow-up (years)	Ventilation support, age at initiation	Age at sitting (months)	Age at independent walking (months)	Baseline LVMI (g/m^2)/ LVMI z-score
1	M	c.1465G > A/·c.40_47del	+(d)	3.1	3.8	No	18.4	No	9.7	16	152/NA
2	F	c.1933G > A/ c.1564C > G	+(d)	3.3	3.7	Yes	16.6	No	5.0	13	NA
3	M	c.1802C > G/·c.2800-1G > C	+(d)	2.9	2.9	No	13.7	Yes, 0.8 years	7.0	No	221/+10.7
4	M	c.525del/·c.2237G > A	–(d)	3.3	3.3	No	11.5	Yes, 2.6 years	18.3	No	229/+11.2
5	F	c.525del/·c.670C > T	+(p)	11.0	11.1	Yes	10.1	No	5.8	12	237/+13.4
6	M	c.2846T > A/·c.2846T > A	+(f)	0.3	0.4	Yes	9.3	No	5.3	12	94/+3.6
7	F	c.525del/·c.2331+1G > A	–(f)	6.1	6.2	No	1.7	Yes, 0.3 years	18.0	No	212/+10.7
8	F	c.525del/·c.1927G > A	+(p)	2.4	3.0	Yes	6.9	No	6.1	12	54/+5.3
9	F	c.525del/·c.1927G > A	+(p)	2.4	3.2	Yes	4.8	No	7.9	14	132/+6.0
10	F	c.1962_1964del/·c.1962_1964del	+(p)	3.7	3.7	Yes	4.7	No	8.0	18	101/+4.3
11§	M	c.235-247delCCACACAGTGC/·c.235-247delCCACACAGTGC	–(p)	0.2	0.6	Yes	4.7	No	8.6	12	NA [33]
12	F	c.1655T > C/·c.2161dup	+(d)	11.5	12.9	Yes	2.6	No	8.6	20	95/+4.1
13§	F	c.235-247delCCACACAGTGC/·c.235-247delCCACACAGTGC	–(p)	0.0	0.3	Yes	1.9	No	7.4	12	NA [33]

Patients 5 and 12 were diagnosed with classic infantile Pompe disease at 11 and 11.5 months, respectively. Hypotonia was reported since the first months of life, but not initially investigated; they both presented severely ill with HCMP, and ERT was promptly started. CRIM status was determined in fibroblasts (f), peripheral blood (p), or based on the Pompe Variant Database (d); NA=Not Available. §= siblings.

ventricular parameters normalized in all patients with a median time to normalization of 9.3 months of ERT (range 1.3–61.1) (Figure 1d), shorter (5.8 months, range 3.0–10.5) for those started on 40 mg/kg/week rhGAA (p=0.06).

Follow-up ECGs were available in 11 patients. At last assessment, at a median age of 5.1 years (range 1.6–18.4), 4 of 11 patients (36%) presented abnormal ECG findings, with short PR intervals being described in 3, delta waves in 3, abnormal repolarization in 4, and ventricular pre-excitation in 1. Patient 8 suffered from recurrent episodes of supraventricular tachycardia, (SVT) which required multiple lines of acute treatment (intravenous adenosine, amiodarone) and chronic anti-arrhythmic therapy with a combination of amiodarone, flecainide, and beta-blocker. The patient is currently a candidate for cardiac ablation.

Antibody titers and immunomodulation

Antibody levels against rhGAA of all 13 patients are provided in Figure 2. Nine of 13 (69%) patients developed anti-rhGAA antibodies, of which five HSAT (38%).

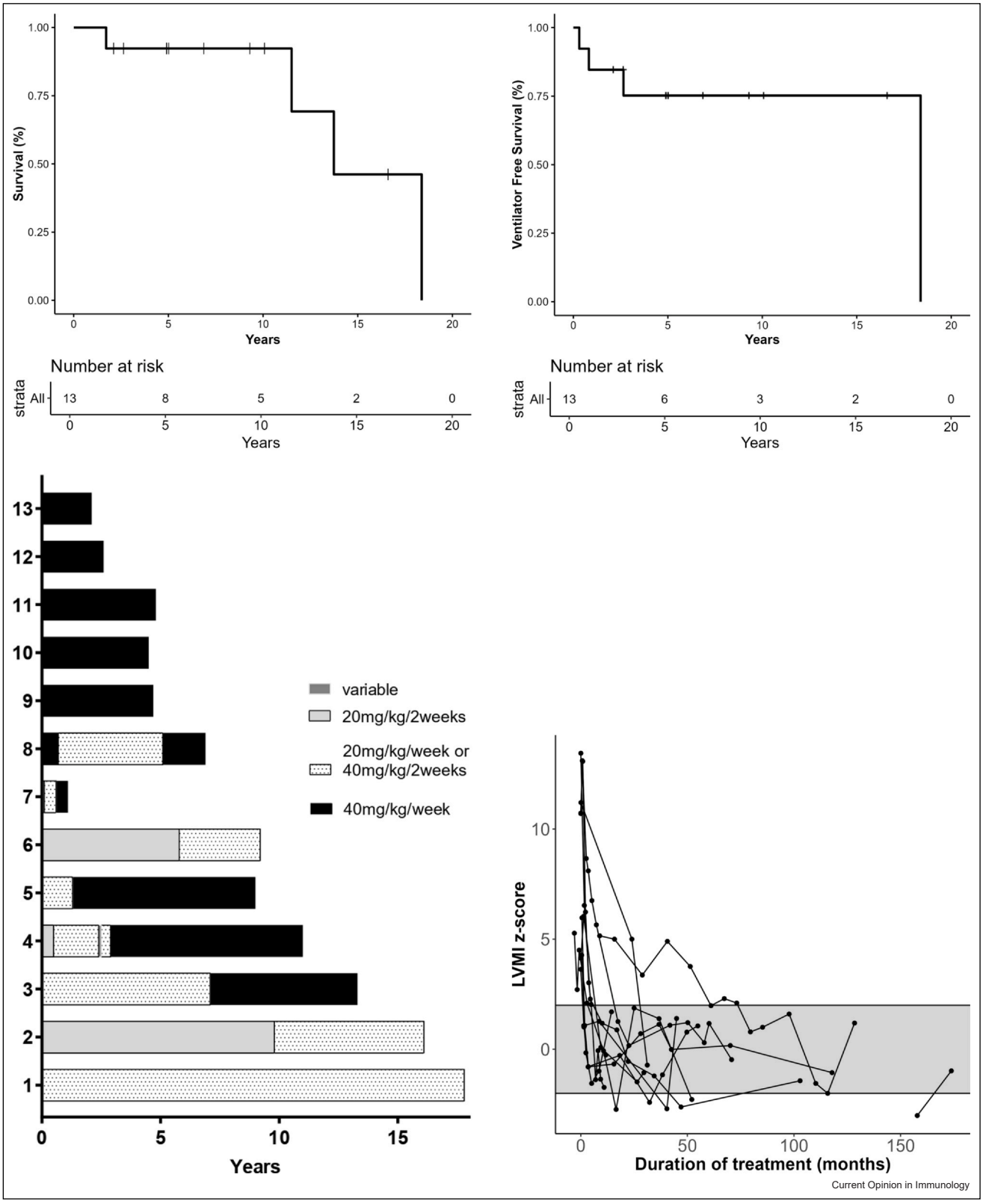
The median time from ERT initiation to first antibody detection was 5.1 months (range 1.7–90.7).

Of the four CRIM negative patients, two developed HSAT (50%) after 0.4 and 0.6 months of treatment, whereas two did not (duration of follow-up 4.7 and 1.9 years). IARs occurred in three patients, all with high antibody titers.

Ten of 13 (77%) patients received ITI, of which five primary, two secondary, and three both primary and secondary (Figure 2, Table 2). Since 2014, primary immunomodulation has been administered to all patients except for one due to bacterial colonization at diagnosis. Each patient was treated with a tailored scheme (Table 2). Subgroup analysis was not possible because of the variability in ITI protocols. No patients experienced serious infections during ITI. Of the five patients who developed HSAT, three had received primary ITI.

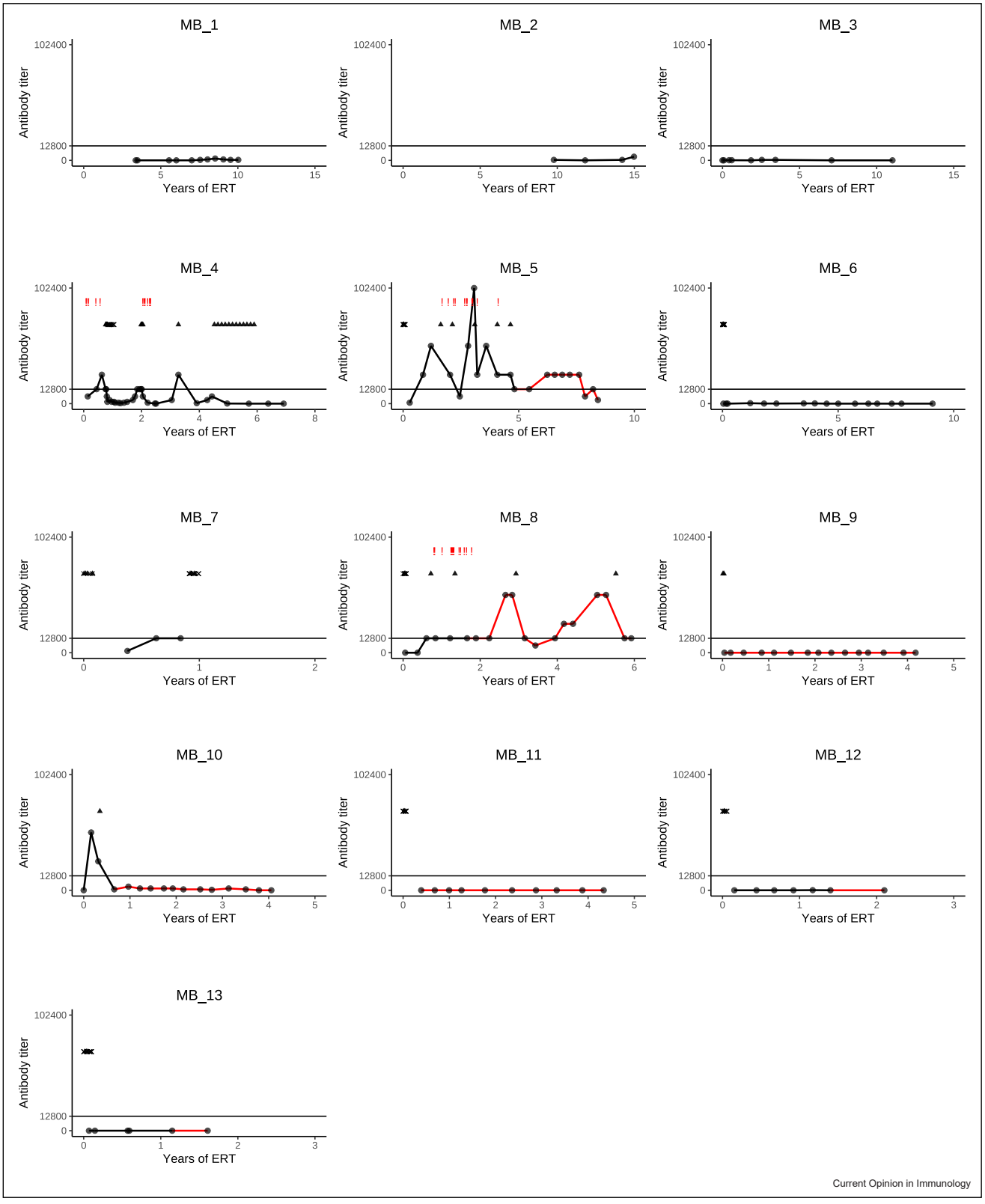
Seven patients were treated with long-term sirolimus (Figure 2, red line). The median interval between the start of ERT and the start of treatment with sirolimus was 6.2 months (range 0.3–57.8). The median duration of treatment with sirolimus was 50.5 months (range 16.8–63.6). Patients 5 and 8 did not present IARs anymore after the introduction of sirolimus. Side effects of sirolimus were mostly represented by small mouth ulcers, which were treated with oral hygiene, topical creams, and lowering the dose to obtain a plasma value of 4–5 ng/mL. Sirolimus was temporarily discontinued only in case of serious illness requiring hospitalization or severe gastroenteritis.

Figure 1



(a) Kaplan–Meier estimates of OS. **(b)** Kaplan–Meier estimates of ventilator-free survival. **(c)** Dose and frequency of ERT with GAA. Each patient is represented by a bar. **(d)** LVMI z-score over months of ERT. The normal range within 2 SDs is represented by the gray rectangle.

Figure 2



Anti-alglucosidase alpha antibody titer. The black horizontal line represents the high antibody titer cutoff (1:12800). Symbols: triangle = rituximab, cross = methotrexate; red line = start of sirolimus; exclamation marks = infusion-associated reactions.

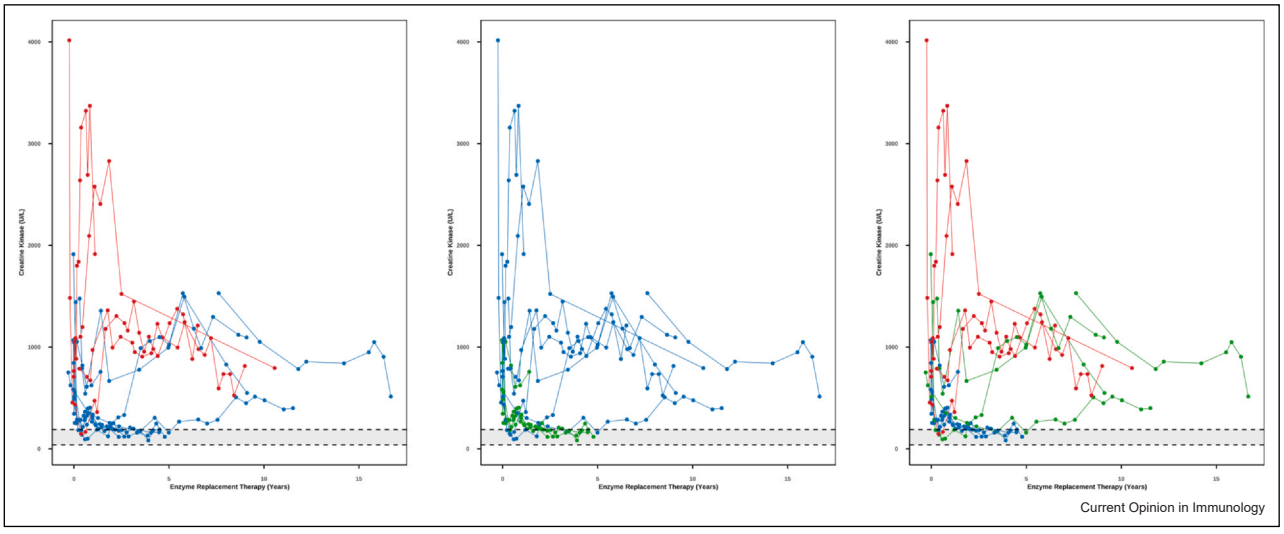
Table 2

Immunomodulation administered to each patient.

PT	CRIM	Primary	Secondary	Sirolimus yes/no (timing of start in months of ERT)	HSAT	IAR
1	+	–	–	No	No	No
2	+	–	–	No	No	No
3	+	–	–	No	No	No
4	–	–	After 9.1 months of ERT, four doses of RTX 375 mg/sqm, weekly MTX 0.4 mg/kg from months 9.9 to 12.5. After 23.8 months of ERT, four doses of RTX 375 mg/sqm. After 39.3 months, one dose of RTX 375 mg/sqm. After 54.2 months of long-term RTX, administered every 8–10 weeks.	No	Yes	Yes
5	+	Banugaria et al. scheme, one dose of RTX not administered	5x single dose of RTX 375 mg/sqm (after 19, 25, 37, 49, 56 months of ERT)	Yes (57 months)	Yes	Yes
6	+	Banugaria et al. scheme	–	No	No	No
7	–	Banugaria et al. scheme, five doses of MTX not administered	After 11 months of ERT, Banugaria et al. scheme. MTX administered orally	No	Yes	No
8	+	Banugaria et al. scheme, one dose of RTX and two doses of MTX were omitted	4x single dose of RTX 375 mg/sqm (after 9, 16, 35, 66 months of ERT)	Yes (18.6 months)	Yes	Yes
9	+	Three doses of RTX 375 mg/mq (administered every 7 days)	–	Yes (0.3 months)	No	No
10	+	–	One dose of RTX 375 mg/sqm (after 4.2 months of ERT)	Yes (4.7 months)	Yes	No
11	–	Banugaria et al. scheme, one dose of RTX not administered	–	Yes (3 months)	No	No
12	+	Banugaria et al. scheme, two doses of RTX not administered	–	Yes (5.6 months)	No	No
13	–	Banugaria et al. scheme	–	Yes (7.8 months)	No	No

The primary immunomodulation scheme adopted has been published by Banugaria et al. [29].

Figure 3



CK values in ERT-treated patients. Gray boxes within discontinued lines represent the normal range. Left panel: the cohort was divided in patients who developed high antibody titers (HSAT, red) and those who did not (blue); Center panel: the cohort was divided in patients who received a ERT regimen of 40 mg/kg/week from start (green) and those who did not (blue); Right panel: the cohort was grouped by ERT regimen from start and antibody status: red = HSAT+ERT regimen different than 40 mg/kg/week; green= no HSAT+ ERT regimen different than 40 mg/kg/week; blue = no HSAT+ERT 40 mg/kg/week from start.

Cross-reactive immunological material-negative patients

Patient 4 was not treated with primary immunomodulation, developed HSAT after 0.4 months of treatment, became ventilator dependent at 2.6 years, and died at 11.5 years. He developed many IARs, which were treated initially with the modification of the ERT regimen, by administering ERT more slowly and over a broader span of time, and even by administering ERT twice a week, then with secondary immunomodulation, which was effective in reducing the antibody titer and eliminating IARs. Patient 7 was severely ill at presentation and was treated with an incomplete primary ITI protocol because of sepsis; she developed HSAT after 0.6 months of ERT, became ventilator-dependent at 0.3 years, and passed away at 1.7 years. Patients 11 and 13 were diagnosed early because of a positive family history (a sibling had deceased of Pompe disease), received primary ITI, and were started on oral sirolimus. At the last follow-up, at ages 4.7 and 1.9 years, they did not develop anti-rhGAA.

Laboratory results

The median creatine phosphokinase (CK) datapoints per patient were 22 (range 8–30) over a median follow-up time of 6.7 years (range 1.1–11.8) (Figure 3).

Data before the start of ERT were available in 12/13 patients; in all cases, it was elevated, and the median CK at diagnosis was 730 U/L (range 455–1913), 3.8 times higher than the upper limit of normal (normal value 40–190 U/L). After starting ERT, in five patients, CK decreased and normalized; in one of the five patients (patient 1), CK subsequently increased at the age of 8.5 years, while in the other four (patients 9, 10, 11, 13), CK was within normal range at last follow-up (median 4.7 years, range 1.9–4.8). Figure 3 (right panel) shows that patients who started ERT at the dose of 40 mg/kg and who never developed a high antibody titer had a lower CK in comparison to the other groups.

Discussion

The aim of this study was to provide the long-term outcome of a cohort of 13 patients with classic infantile Pompe disease receiving rhGAA, in terms of survival, motor and cardiac outcomes, antibody titers, and creatine kinase. The findings were compared to previously published cohorts, summarized in Table 3.

The key features of the Monza cohort include a high 5- and 10-years survival rate (92%), cardiac normalization (100%) and walking achievement (77%), considering a low percentage (30%) of patients receiving the labeled ERT dose at treatment initiation, a high rate of immunomodulation (77%), particularly among CRIM+ naïve patients (55% of CRIM+ subjects), and a low percentage of patients with high titers (38%).

All patients in the Monza cohort were clinically diagnosed with Pompe disease, as it is not yet included in the Italian national newborn screening (NBS) program.

Table 3

Summary of previously published outcomes of international cohorts of infantile Pompe disease patients treated with recombinant human alglucosidase alpha and the Monza cohort.

Author	Year of publication	NBS Yes/ No (% of pt)	No. treated pt	% CRIM+/ CRIM– / NA	Median age start ERT, months (range)	Median age last visit, years (range)	% pt treated with label dose from start	% HSAT	% ITI (P, S, P+S)	10-year OS and VFS	% pt achieving walking, median age in months (range)	% pt with HCMP at last follow-up
Tardieu et al.	2023	No	50	72% / 26% / 2%	4.0 (0–15)	2 (0–15.5)	60%	NA	26% (P 18%, S 8%)	OS 50% VFS 30%	28%, 18 months (12–65)	56%
Pfrimmer et al.	2024	No	15	86%/ 7%/ 7%	5.0 (1.5–31.0)	9 (7.0–19.5)	80%	13%	20% (P 7%, S 13%)	OS NAVFS 55%	47%, 24 months (14.4–26.4)	0%
Parini et al.	2018	No	28	61% / 25% / 14%	4 (0–11)	6 (2–11.5)	93%	38%	10% (P 3%, S 7%)	OS 50% VFS 20%	25%, 16.5 months (12–19)	47%
Poelman et al.	2020	No	18	72% / 28%	3.1 (0–6)	4.4 (0.6–12.6)	33%	50%	44% (P 22%, S 17%, P+S 5%)	OS 83%VFS 50% label dose, 92% 40 mg/kg/week	83%, NA	11%
Li et al.	2021	7 (35%)	20	0% / 100%	Range 0–7	Range 0.7–11.2	90%	10%	100% (P 100%, S 0%)	OS 82% VFS 62%	60%, NA	35%
Banugaria et al.	2011	No	34	67% / 33%	Range 0–15	< 4 years	NA	54%	0%	NA	NA	NA
Yang et al.	2022	Yes	26	100% / 0%	9.7 days (6–18)	5.0 (0–10.4)	100%	NA	0%	OS 100% VFS 100%	100%, mean age 11.9 months ± 1.0	NA
Chien et al.	2015	Yes	10	100% / 0%	16 days (6–34)	5.2 (2.3–7.6)	100%	10%	0%	OS 100% VFS 100%	100%, 14 months (12–20)	0%
EPOC	2022	No	116	63% / 20%/ 17%	3.3 (0–11.8)	Mean 5 (± 4.7)	41%	NA	NA	OS 63% VFS NA	51%, 16.0 months (11–39)	NA
Pompe Registry	2023	51 (15.4%)	332	64% / 21%/ 15%	3.6 (0.1–11.6	NA	81%	NA	20%	OS 74% VFS 64%	NA	NA
Monza	–	No	13	69% / 31% / 0%	3.3 (0.3–12.9)	6.9 (1.9–18.4)	30%	38%	77% (P 38%, S 15%, P+S 23%)	OS 92% VFS 75%	77%, 12.5 months (12.0–20.0)	0%

Abbreviations: EPOC = European Pompe Consortium, PT = patient; NA= not available; P =primary (naïve setting); S = secondary (in case of HSAT); P+S = both primary and secondary; VFS = ventilator free survival.

The median age at ERT initiation was 3.3 months, comparable to that reported in larger cohort studies [17,18]. CRIM– patients accounted for 31% of the cohort, a proportion consistent with European and American populations [9–11,14,17,18], but differing from the Taiwanese group, which is predominantly CRIM+ [35].

OS at 5- and 10- years was 92%, higher than data reported in larger cohorts (50% at 10 years in the French cohort [11], and 60% in the European Pompe Consortium [17] population), although only five Monza patients had a follow-up longer than 10 years; the higher survival of this population probably reflects the younger population, which is currently treated with higher doses of ERT and immunomodulation. In fact, similarly to recent reports [9,11,17,18], only 30% of this cohort was started on treatment at the standard dose of 20 mg/kg/2 weeks, and two out of the four patients were switched to a higher dose within 3 months of the start of ERT.

In the Monza cohort, all patients learnt to sit without support; 10 of 13 (77%) acquired ambulation, and one patient subsequently lost the ability to walk. Achievement of the walking milestone in this cohort was higher in comparison to the cohort from Germany-Austria (47%) [9], France (28%) [11], and the European Consortium (51%) [17]. On the contrary, 100% of Taiwanese patients detected by NBS who started ERT at a median age of 16 days achieved walking [35]. All subjects who were treated with rhGAA at a dose of 40 mg/kg/week from the start achieved the walking milestone, in comparison to four of the seven remaining patients. Although limited by the small sample size, such a result is aligned with the most recent large cohort population studies [17,18], which confirm a higher survival and better motor outcome with high ERT doses.

All patients presented heart involvement at baseline, with a median LVMI z-score of +6 SDs, similar to data reported by Capelle et al [30]. Left ventricular dimensions normalized in all cases after a median duration of ERT of 9.3 months, in line with previously described cohorts (7.5–12 months) [30,36], confirming the rapid and optimal response of cardiomyocytes to rhGAA. Similar satisfactory results have been reported by the German–Austrian [9] and Dutch cohort [30], while in the French cohort, of which 60% was initiated on the licensed dose of rhGAA, there was a high persistence (56% of cases) of cardiomyopathy at the last visit, but at a relatively young age (median age 24 months, range 1–186 months). While cardiac hypertrophy responds well to ERT, 36% of the Monza cohort still presented electrical abnormalities at ECG, and one patient especially suffered recurrent episodes of SVT, for which she is candidate to cardiac ablation; in the German–Austrian cohort 50% of patients either had SVT or Wolff-Parkinson-White pattern (WPW) [9], while in the Dutch cohort, 57% of patients at end of follow-up presented a short PR interval, 42% a WPW pattern and three patients developed spontaneous SVT episodes requiring medical intervention [30]. The underlying mechanism for the persistence of ECG abnormalities is not yet fully understood. It could potentially be explained by the presence of glycogen within the conduction system, or by the structural changes induced by its deposition, such as cardiomyocyte enlargement [37,38]. CK has not been considered a reliable biomarker in Pompe disease, as it is influenced by multiple factors: physical exercise and viral infections can lead to an increase, while a good response to therapy as well as immobility and muscle atrophy may lead to lower values. In patients with the classic infantile, form CK is usually increased both at diagnosis and during follow-up [3] [9]; nevertheless, it can normalize in case of young age at start of treatment, immunomodulation, and high doses of ERT. In 10 patients detected by NBS with high CK baseline values, CK decreased after starting ERT at a dose of 20 mg/kg/2 weeks to a nadir at age 6 months of life. However, it increased gradually thereafter, resulting in elevated levels in all patients at age 5 [35]. Li et al. reported normal or near normal CK values over time in four of five CRIM negative patients who received primary immunomodulation and who had started ERT within 4 weeks, while it was elevated in CRIM– subjects who received immunomodulation but started ERT later [39] and Chien et al. observed that patients who were started on higher doses of ERT presented lower CK values [40]. In the Monza cohort, CK was elevated at diagnosis in all patients. We identified a subgroup of four subjects in whom CK normalized after the start of ERT and remained within normal range; these four patients were treated since diagnosis with a weekly 40 mg/kg rhGAA dose, received primary ITI, have not developed high antibody titers yet (median follow-up 4.7 years, range 1.9–4.8), and present a normal motor development. Two of these four patients are CRIM–. Follow-up is still short, and we cannot exclude that CK will later increase, as occurred in a fifth patient. From this limited data, CK seems to be a good biomarker for therapy response and requires more attention. Such findings should be assessed in larger cohorts as well as correlated to clinical outcome parameters such as 6-minute walking test and forced vital capacity.

An initial aim of this study was to assess the effect of immunomodulation on antibody titers. However, this was not feasible because the treatment regimens varied substantially among patients, ranging from full protocols to single doses of individual agents. Nevertheless, the analysis provided valuable insight into the 'real-world' management of classic infantile Pompe disease.

In our center, immunomodulation has always been decided in a multidisciplinary team with hematologists and immunologists. The scheme of Banugaria et al. was more frequently adopted as the primary

immunomodulation scheme in 7 of 13 patients, even though it was administered entirely only in two of seven. In fact, in the other five cases, doses of immunomodulators were omitted because of fever or leukopenia. In terms of secondary immunomodulation for sustained high antibody titers, mostly single doses of rituximab were administered, chosen as a risk-benefit balance, in contrast to the experience of other centers, where, in the last few years, long-term immunomodulation seems to be more widely adopted, consisting of a period of 4 weeks during which methotrexate, rituximab, bortezomib are administered, followed by long-term rituximab, prescribed every 4–10 weeks [20,24]. In our experience, we observed, as shown in Figure 2, that a single dose of rituximab was efficacious in reducing antibody titers, but temporarily, as in most cases (except patient 10), the titer later increased. To overcome this, our center could implement ITI with long-term rituximab or with a combination of drugs.

Overall, 77% of patients in Monza received ITI, a much higher percentage in comparison to the French cohort (27%) [11] and German–Austrian one (20%) [9]. Primary ITI was administered to five out of nine (55%) CRIM+ ERT-naïve patients, and it has been considered in all patients since 2014. This approach was implemented after experiencing the challenges in eliminating HSAT in ERT-experienced subjects, in line with recent evidence showing that early intervention reduces the overall need for immunomodulatory treatment compared to initiating it only after antibody development [24,41].

A total of 69% of patients developed anti-rhGAA antibodies, and 38% HSAT, a remarkably low percentage in comparison to other cohorts [14,21]. Three of eight subjects who received primary ITI developed HSAT, for which secondary ITI was administered, revealing that primary ITI does not fully eliminate the risk of developing HSAT. Three of five patients with HSAT developed IARs, which did not occur in those without HSAT; in all cases, secondary ITI was administered.

Focusing on CRIM status, three of the nine (33%) CRIM+ patients developed HSAT, despite two receiving primary ITI. Additionally, two out of four (50%) CRIM– patients presented with HSAT, one of whom did not receive primary ITI. The other two CRIM– patients, patients 11 and 13, were diagnosed early because of a positive family history, treated with primary ITI, and started treatment with oral sirolimus. At the age of 1.9 and 4.7 years, these patients have not developed anti-ERT antibodies, have a normal motor development, and normal CK values. The 'real world' data reported shows that the introduction of immunomodulation protocols has modified the acquired knowledge on anti-ERT antibodies, revealing that, although CRIM negative status still represents a risk factor for a worse outcome, timely diagnosis and ITI protocols can eliminate such risk excess. In the French cohort, CRIM status was not related to survival [11]; the OS at 10 years in another CRIM– cohort of 20 subjects, who received primary ITI, was 70%, greatly improved in comparison to a historical CRIM– cohort who did not receive primary ITI and had all deceased by age 50 months [39].

These observations underline that, despite the timely start of ITI, a substantial proportion of both CRIM– and CRIM+ patients still develop HSAT. This ongoing challenge has provided the rationale for introducing additional immunomodulatory strategies, including sirolimus, an inhibitor of the mammalian target of rapamycin (mTOR) pathway, which has been increasingly adopted by our center for classic infantile Pompe patients, for two reasons: 1) as a immunomodulator cofactor working on T cell memory and 2) as a potential therapeutical cofactor acting as an inhibitor of mTORC1. Sirolimus, in fact, blocks T-cell activation by inhibiting dendritic cells maturation and selectively allows for stimulation of antigen-specific Foxp3+ regulatory T-cells [42]. Beyond its immunological effects, sirolimus may influence glycogen accumulation, the central pathogenic mechanism in Pompe disease, which triggers many secondary processes, such as impaired autophagy, mitochondrial dysfunction, disturbed calcium homeostasis [43,44], and ultimately muscle atrophy [45]. mTOR is a master regulator of cellular growth and metabolism, essential for muscle mass homeostasis. Activation of mTORC1 promotes protein synthesis and cell growth while suppressing autophagy, with the lysosome serving as a key activator [46]. In the Pompe mouse model, mTOR dysfunction has been described as a consequence of defective lysosomal acidification due to glycogen accumulation [46]: mTOR activity is diminished, less responsive to starvation, and misplaced within skeletal myocytes. Autophagy impairment with accumulation of autophagic debris has been observed [45]. Pre-clinical studies have suggested that sirolimus reduces glycogen accumulation by inhibiting mTORC1 and increasing phosphorylation of glycogen synthase in skeletal muscle [42,47], without affecting glucose homeostasis. In GAA-KO mice, Lim et al. demonstrated that combining ERT with synthetic vaccine particles carrying sirolimus (SVP-Rapa) enhanced glycogen clearance and delayed development of anti-rhGAA compared to ERT alone or ERT+MTX [42]. Clinical experiences in classic infantile Pompe patients have yielded mixed results. Elder et al. combined rituximab with mycophenolate or sirolimus for B cell depletion and T cell modulation prior to ERT in five patients (four CRIM- and one CRIM+). One CRIM- patient developed HSAT upon B-cell recovery, with subsequent clinical decline and death, while ITI successfully prevented antibody formation in the remaining four [48]. Poelman et al. used a regimen including bortezomib,

rituximab, sirolimus, and IVIG in patients who developed HSAT. Secondary immunomodulation reduced antibody titers and prevented further infusion reactions, but did not eliminate IgG antidrug antibodies or induce immune tolerance [26].

In the Monza cohort, sirolimus was started early (median 6.2 months after ERT initiation). It was well tolerated with no increased infection risk, but its effect on clinical outcome could not be determined. Two patients (patients 5 and 8) presented IARs, despite rituximab, which disappeared after adding sirolimus. However, sirolimus did not prevent HSAT formation in these two subjects. Conversely, four patients with low titers started sirolimus without subsequently developing HSAT. Given the complex interplay of multiple factors, it remains difficult to attribute outcomes to sirolimus alone. Rigorous clinical studies, alongside studies on biomarkers and/or proteomics in sirolimus-treated versus untreated patients, are needed to clarify its effect on muscle dysfunction and antibody formation.

This study presents numerous limitations due to the rare disease and real-world setting, leading to a small sample size and heterogeneity in enzyme replacement regimen and immunomodulation protocols. Additionally, no data concerning motor outcome parameters were reported. Nevertheless, it provides a comprehensive insight into the actual clinical management of patients with classic infantile Pompe disease, providing detailed cardiac parameters, as well as longitudinal creatin kinase and antibody titer follow-up. The intertwined impact on the outcome of ERT dosage and immunomodulation is highlighted and underlines the difficulty in analyzing single factors in real-world practice. Finally, we provide follow-up of the probably largest cohort of classic infantile Pompe patients receiving sirolimus.

Conclusion

The main characteristics of the Monza cohort in comparison to previously published cohorts include a high 5- and 10-years survival rate (92%), cardiac normalization (100%) and walking achievement (77%), considering a low percentage (30%) of patients receiving the labeled ERT dose at treatment initiation, a high rate of immunomodulation (77%), particularly among CRIM+ ERT-naïve patients (55% of CRIM+ subjects), and a low percentage of patients with high titers (38%). The Monza group was similar in terms of age at start of treatment and CRIM– population (31%) to other European and American cohorts. All patients presented with elevated CK at diagnosis. We identified a subgroup of subjects who normalized and maintained normal CK values with a follow-up of 4.7 years. Sirolimus was widely used in our center and was well tolerated by patients. Cardiac rhythm abnormalities and high-sustained antibody titers remain important challenges, and the role of sirolimus requires further study.

Ethics approval and patient consent statement

The study was approved by the Pediatric Italian National Ethical Committee, protocol number 198. Written consent was obtained from the patients/legal guardians.

CRediT authorship contribution statement

M.C.F. and V.C. designed the study, collected and analyzed the data, and wrote several drafts of the manuscript. G.R. supported statistical analysis, figure optimization, and reviewed the manuscript. A.T.P. and J.M.P.H. contributed to data interpretation and analysis and reviewed several drafts of the manuscript. R.P., G.K., L.T., K.P., S.B., M.S., A.B. contributed to results discussion and draft revision. L.B. revised all cardiological data and reviewed the manuscript. S.G. participated in the study design, data interpretation and analysis, graphical visualization, and reviewed several drafts of the manuscript.

Funding

This article has been partially funded by the Italian Ministry of Health, 'Ricerca Corrente' Program.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank Vera Marchetti for her great work, all the nurses of the Pediatric Day Hospital (especially Katia Pozzi, Silvia Gigante, Alessia Cerizza), Fondazione Mariani for its support to research and improvement of quality of life of patients and their families, and the patients and their families for participating in this study. Several of the authors of this publication are members of the European Reference Networks for Hereditary Metabolic Disorders (Metab-ERN). This article has been partially funded by the Italian Ministry of Health, 'Ricerca Corrente' Program.

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