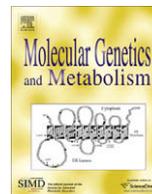




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Cross-reactive immunologic material status affects treatment outcomes in Pompe disease infants

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ABSTRACT

Deficiency of acid alpha glucosidase (GAA) causes Pompe disease, which is usually fatal if onset occurs in infancy. Patients synthesize a non-functional form of GAA or are unable to form native enzyme. Enzyme replacement therapy with recombinant human GAA (rhGAA) prolongs survival in infantile Pompe patients but may be less effective in cross-reactive immunologic material (CRIM)-negative patients. We retrospectively analyzed the influence of CRIM status on outcome in 21 CRIM-positive and 11 CRIM-negative infantile Pompe patients receiving rhGAA. Patients were from the clinical setting and from clinical trials of rhGAA, were ≤ 6 months of age, were not invasively ventilated, and were treated with IV rhGAA at a cumulative or total dose of 20 or 40 mg/kg/2 weeks. Outcome measures included survival, invasive ventilator-free survival, cardiac status, gross motor development, development of antibodies to rhGAA, and levels of urinary Glc₄.

Following 52 weeks of treatment, 6/11 (54.5%) CRIM-negative and 1/21 (4.8%) CRIM-positive patients were deceased or invasively ventilated ($p < 0.0001$). By age 27.1 months, all CRIM-negative patients and 4/21 (19.0%) CRIM-positive patients were deceased or invasively ventilated. Cardiac function and gross motor development improved significantly more in the CRIM-positive group. IgG antibodies to rhGAA developed earlier and serotiters were higher and more sustained in the CRIM-negative group. CRIM-negative status predicted reduced overall survival and invasive ventilator-free survival and poorer clinical outcomes in infants with Pompe disease treated with rhGAA. The effect of CRIM status on outcome appears to be mediated by antibody responses to the exogenous protein.

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Introduction

Pompe disease (glycogen storage disease type II) is a lysosomal storage disorder resulting from deficiency of acid alpha glucosidase (GAA). This deficiency causes lysosomal accumulation of glycogen in cardiac, skeletal, and smooth muscle tissue, resulting in progressive cardiac, motor, and respiratory failure. The infantile pheno-

type is characterized by rapidly progressive muscle weakness, hypertrophic cardiomyopathy, feeding difficulties, and eventual respiratory insufficiency. Patients usually present with disease symptoms at approximately 3 months of age and death occurs at a median age of 6.0–8.7 months [1,2]. The reported frequency of infantile-onset Pompe disease ranges from 1 in 33,333 in Taiwanese populations to 1 in 138,000 in Dutch populations [3,4]. Development of Chinese hamster ovary- (CHO) and transgenic rabbit milk-derived enzyme replacement therapy (ERT) paved the way for the first available targeted therapy for Pompe disease [5,6]. Clinical trials of ERT showed a positive response in infantile Pompe patients [7–12]. CHO-derived rhGAA (alglucosidase alfa; Myozyme[®]) was approved in the USA, Europe, and Canada in 2006, with subsequent approvals in numerous countries worldwide.

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¹ Statements made in this paper represent the opinions of Dr. Rosenberg and not necessarily those of the US Food and Drug Administration.

Timely intervention with ERT is essential to halt motor disease progression and reverse cardiomyopathy in infantile Pompe patients; response is more attenuated if ERT is initiated when the disease is more advanced [12]. Despite early treatment, however, some patients with infantile Pompe disease respond poorly to ERT. Factors that are important in determining outcome include extent of pathology and muscle damage at the time of start of ERT [9,11,13]. However, these factors do not sufficiently predict disease course or response to ERT.

In the first clinical trial using CHO cell-derived rhGAA, we suggested that the presence or absence of cross-reactive immunological material (CRIM) may affect prognosis [8]. Patients with two deleterious GAA mutations who are completely unable to form native enzyme are CRIM-negative; patients with presence of some residual, functioning or non-functioning enzyme are CRIM-positive. In this pilot trial the two patients who were CRIM-negative died, while the single patient who was CRIM-positive had a very good motor response and is currently alive and ambulatory [8]. Concomitant with clinical decline, persistent high anti-rhGAA IgG antibody titers were found in the two CRIM-negative patients while titers for the CRIM-positive patient remained low. Similar observations from our group were reported in a subsequent clinical trial [9]. However, this correlation was not confirmed by others [7,14]. CRIM-positive patients tend to have low antibody titers, which are associated with the best response to ERT.

To gain a better understanding of the role of CRIM status as a prognostic factor, we conducted a retrospective analysis comparing outcomes of CRIM-positive with outcomes of CRIM-negative patients with Pompe disease from the clinical setting and from clinical trials of rhGAA. Subjects were age 6 months or younger at the initiation of ERT. Inclusion and exclusion criteria and endpoints were similar to those used in the pivotal trial of alglucosidase alfa [11]. We hypothesized that CRIM-negative patients would have a poorer clinical outcome compared to CRIM-positive patients. Primary clinical outcomes selected were survival, ventilator-free survival, and cardiac and motor response, corresponding with the key endpoints in the pivotal trial of alglucosidase alfa [11]. Additional analyses included urinary Glc₄ levels and antibody titers.

Methods

Study design

Retrospective data were collected from clinical trials of CHO-derived rhGAA conducted between 1999 and 2006 [8,9,11,12] and from a retrospective chart review of several clinical patients who met inclusion and exclusion criteria and were part of expanded access programs at two sites. Inclusion and exclusion criteria were chosen to resemble those for the pivotal trial of alglucosidase alfa [11]. Principal inclusion criteria were: confirmed diagnosis of Pompe disease; age at enrollment ≤ 6 months by adjusted gestational age; $< 1\%$ of normal GAA activity (in skin fibroblasts and/or muscle biopsy); and cardiomyopathy (left ventricular mass index [LVMI] ≥ 65 g/m² by echocardiogram). Exclusion criteria included respiratory insufficiency, major congenital anomaly, or any prior rhGAA treatment. Parents or guardians of all patients in these studies gave consent to institutional IRB- or ethics committee-approved protocols. All patients received rhGAA supplied by Genzyme Corporation (Cambridge, MA) at a cumulative or total dose of 20 or 40 mg/kg every 2 weeks according to previously published reports [8,9,11,12].

Clinical outcomes

Survival and invasive ventilator-free survival were analyzed in the original clinical trials; we summarize these data and present

updated survival data through June 2006, at which time the database was locked. Patients included in this analysis from the clinical setting were followed until age 18 months, as done for the pivotal trial of alglucosidase alfa [11]. Two-dimensional, M-mode, and Doppler echocardiography were used to assess LVM index at baseline, 26, and 52 weeks after the initiation of ERT. Motor function evaluation was performed using the Alberta Infant Motor Scale (AIMS) [15] by an experienced physical therapist at baseline, 26, and 52 weeks after the initiation of ERT.

Laboratory methods

Cultured skin fibroblasts obtained from Pompe patients were assayed for GAA enzyme activity using 4-methyl-umbelliferyl- α -D-glucopyranoside (4MUG) as an artificial substrate described in Kishnani et al. [9].

CRIM status was determined as described previously [9,16] based on reactivity of a pool of monoclonal or polyclonal antibodies generated against GAA that could recognize both native and recombinant forms of GAA. Briefly, cell lysates derived from patient fibroblast cells were subjected to Western blot analysis using a 4–12% gradient gel and a pool of monoclonal antibodies (generated against rhGAA) and a polyclonal antibody that was generated against human placental GAA, which recognizes both precursor and processed forms of GAA protein. A patient was designated as CRIM-positive if any of the GAA protein forms (unprocessed precursor band at 110 kDa or any of the processed forms) were detectable on the Western blot analysis; a patient was designated as CRIM-negative if none of these protein forms was detectable on Western blots (processed and unprocessed). Results were initially generated by one laboratory and were confirmed by a second, independent laboratory (one using monoclonal antibodies, the other polyclonal antibodies) in a blinded fashion. Representative Western blot showing CRIM-positive and CRIM-negative patients is shown in Fig. 1.

In patients whose parents provided informed consent for genotyping, DNA was isolated from peripheral blood and sequenced. GAA mutation analysis was determined by Genzyme Corporation, as described previously [9,11].

Anti-rhGAA IgG antibodies were assessed at baseline and at weeks 4, 8, 12, 24, 38, and 52. In surviving infants, serotiters were followed up to 80 weeks. Antibody status was ascertained using enzyme-linked immunosorbent assays and confirmed using radio-immunoprecipitation, as previously described [9]. Patients from the first trial of three patients [8] were excluded from antibody

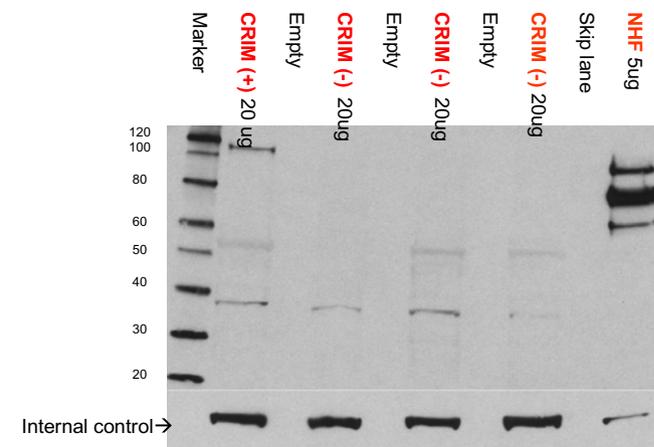


Fig. 1. Representative gel showing CRIM-positive and CRIM-negative samples run on the gel with known positive and negative control samples.

analyses. Additional testing to determine the presence of inhibitory antibodies towards enzyme uptake or enzyme activity was performed in patients according to the respective clinical trial protocols and the requirements of the Genzyme Pharmacovigilance department. An inhibitory antibody assay (enzyme activity) was used to measure inhibition of rhGAA enzyme activity by antibodies present in patient serum and a flow cytometry-based assay (enzyme uptake) was used to evaluate whether patient antibodies interfere with uptake of rhGAA by human fibroblast cells in culture.

Urine oligosaccharides were obtained to assess and follow Glc₄, a biomarker for Pompe disease, at baseline and at weeks 4, 12, 26, 38, 52, 64, and 78. Urinary Glc₄ was measured by HPLC–UV and tandem mass spectrometry (ESI-MS/MS) as previously described [17,18].

Statistical analysis

Survival data were analyzed using the Kaplan–Meier method [19] with two-tailed *p*-values generated using the log-rank test. Other reported *p*-values were generated by the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables. Analyses were performed with STATA version 9.0 (StataCorp LP, College Station, Texas). Due to the limited sample size, all group outcome variable data are presented as medians.

Results

A total of 32 patients from the source clinical trials and from a subset of infantile patients at 2 sites who met inclusion and exclusion criteria for this analysis: 11 CRIM-negative patients and 21 CRIM-positive patients. Baseline demographics and disease-related characteristics (age, LVM index, AIMS score, and urinary Hex 4 levels) were comparable between the two groups (Table 1). Genotypes of CRIM-negative patients who consented to genotyping are presented in Table 2.

Survival

Ventilator-free survival was significantly better for the CRIM-positive group than the CRIM-negative group [Fig. 2; *p* < 0.001].

Table 1
Baseline demographics.

	CRIM-negative N = 11	CRIM-positive N = 21
<i>Gender</i>		
Male	5 (45%)	15 (71%)
Female	6 (55%)	6 (29%)
<i>Race</i>		
White	4 (36%)	10 (48%)
Black	3 (27%)	2 (10%)
Hispanic	1 (9%)	3 (14%)
Asian	1 (9%)	5 (24%)
Other	2 (18%)	1 (5%)
<i>Age at Symptom Onset (months)</i>		
Mean (SD)	1.23 (0.9)	1.67 (1.60)
Median	1.45	1.50
Min, Max	0, 2.4	0, 5.4
<i>Age at First ERT (months)</i>		
Mean (SD)	3.55 (1.93)	4.59 (2.05)
Median	3.00	5.10
Min, Max	0.25, 7.0	0.25, 7.3
<i>Adjusted Age at First ERT (months)</i>		
Mean (SD)	3.19 (1.81)	4.20 (1.81)
Median	3.15	4.8
Min, Max	0, 6.1	0, 6.1

After 52 weeks of ERT, 6/11 (54.5%) CRIM-negative patients were either deceased (*n* = 1) or on invasive ventilation (*n* = 5) as compared to only 1/21 (4.8%) CRIM-positive patient, who was ventilator dependent. By 27.1 months of age, all 11 CRIM-negative patients were deceased (*n* = 5) or on a ventilator (*n* = 6), compared to 4/21 (19.0%) CRIM-positive patients (*n* = 1 deceased, *n* = 3 invasively ventilated).

Cardiac function: left ventricular mass index

The upper limit of normal LVMI for infants is 64 g/m² [20]. In this study, both groups showed similarly elevated LVMI at baseline [median LVMI 202.1 g/m² for CRIM-negative patients (*n* = 10) and 207.8 in CRIM-positive patients (*n* = 19) (*p* = 0.96)]. After 26 weeks of rhGAA, both groups had a net decrease in LVMI (Fig. 3A). In marked contrast, at 52 weeks, CRIM-positive patients (*n* = 18) demonstrated additional reduction of median LVMI to near-normal levels (LVMI = 63.9 g/m²), while median LVMI in surviving CRIM-negative patients (*n* = 9) increased to 129 g/m² (*p* = 0.0005).

Gross motor development

At baseline, gross motor development was delayed and essentially the same for both groups. The average age of CRIM-negative (*n* = 10) and CRIM-positive (*n* = 21) patients at ERT initiation was 3.6 months and 4.6 months, respectively; the average score on the Alberta Infant Motor Scales was 5.2 (age equivalent [AE] = 3 weeks) and 7 (AE = 4 weeks), respectively. After 26 weeks of ERT, gross motor function improved in both groups, but the median AIMS score for the CRIM-negative group (10) was below that for the CRIM-positive group (25; *p* = 0.07) (Fig. 3B). After 52 weeks of ERT, the median AIMS score for CRIM-negative patients (5.5; AE = 3 weeks) was less than the median score for the CRIM-positive patients (48.5; AE = 10 months; *p* = 0.006).

Anti-rhGAA antibody determination

All CRIM-negative patients (*n* = 8) and 18/20 (90%) CRIM-positive patients who were tested developed IgG antibodies to rhGAA. All CRIM-negative patients seroconverted by 4 weeks of ERT. The average time of seroconversion in the CRIM-positive group following ERT initiation was 12.7 weeks (Table 3). Serotiters were significantly different at 24 weeks (median titer of 1:51,200 CRIM-negative and 1:600 CRIM-positive; *p* = 0.0010) and remained significantly different at 52 weeks (median titer of 1:153,600 CRIM-negative and 1:200 CRIM-positive; *p* = 0.0010).

Titers were generally higher in the CRIM-negative patients, whose median peak titer level across subjects was 1:204,800 compared with a peak titer of 1:1800 in CRIM-positive patients. Of the 15 CRIM-positive patients who had seroconverted and for whom antibody data were available at 52 weeks, 9 (60%) had titers less than or equal to 1:800 for that time point and all subsequent time points, while 6 (40%) had titers of 1:3200 or higher at 52 weeks (range 1:3200–1:51,200), with subsequent titers staying at or above 1:1600. Conversely, the CRIM-negative group showed a continued increase in serotiters, effectively tripling median titers from 1:51,200 at 24 weeks to 1:153,600 at 52 weeks of ERT. No CRIM-negative patient showed a consistent decline in titer levels and all CRIM-negative patients developed titers of at least 1:25,600.

Additional testing to detect inhibition of enzyme uptake or inhibition of enzyme activity was conducted on samples from most patients who developed anti-rhGAA antibodies and experienced infusion-associated reactions (IARs) or showed signs of clinical decline. Twenty two patients (5 CRIM-negative, 17 CRIM-positive) were tested for activity inhibition and 19 patients (5 CRIM-negative, 14 CRIM-positive) were tested for uptake inhibition. None of

Table 2
GAA gene mutations in 9/11 CRIM-negative patients.

Race	Maternal allele	Paternal allele
Caucasian	c.722–723 del TT (frameshift)	c.1687C > T (p.Gln563Stop)
African American	c.2560C > T (p.Arg854Stop)	c.2560C > T (p.Arg854Stop)
Asian	c.148_859–11del (p.Glu50 fs, del exons 2–4) null allele	686insCGGC (p.Arg229fs)
Arab	c.2560C > T (p.Arg854Stop)	c.2560C > T (p.Arg854Stop)
Israeli	c.1075G>A (p.Gly359Stop)	c.1075G>A (p.Gly359Stop)
African American	c.2560C > T (p.Arg854Stop)	c.2560C > T (p.Arg854Stop)
Caucasian	c.1754 IVS12 + 1G > A (splicing)	c.722_723delTT(p.Phe241Cys fs-Ter 88)
Arab	c.1209delC (p.Asn403Lysfs-Ter37)	c.1209delC (p.Asn403Lysfs-Ter37)
Hispanic	c.1496 G > A (p.Trp498Stop)	c.1496 G > A (p.Trp498Stop)

Kroos et al. [42].

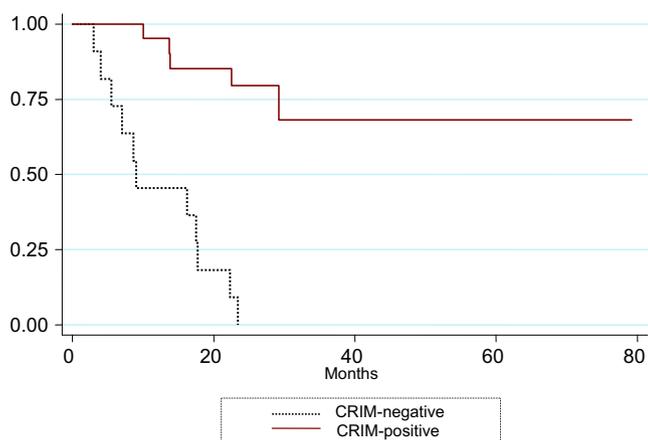


Fig. 2. Kaplan–Meier curve of ventilator-free survival of the CRIM-negative ($n = 11$) and CRIM-positive ($n = 21$) patients.

the CRIM-positive patients tested showed detectable levels of uptake or activity inhibition. Three of the five CRIM-negative patients tested positive for uptake inhibition; two of those patients also tested positive for activity inhibition. Five CRIM-negative and 4 CRIM-positive patients in the current study were never tested for activity or uptake inhibition, and an additional 3 CRIM-positive patients were never tested for uptake inhibition.

Biomarker Glc_4

There was no significant difference in urine Glc_4 levels between the two groups at baseline ($z = -0.9032$; $p = 0.3667$). After 52 weeks of ERT, Glc_4 levels for CRIM-positive patients remained low (median Glc_4 level = 15.0 mmol/mol creatinine), while Glc_4

levels in the CRIM-negative group increased (median Glc_4 level = 42.9); the difference at 52 weeks was significant ($z = 2.829$; $p = 0.0047$; Fig. 3C).

Discussion

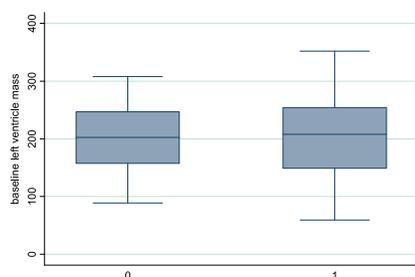
ERT with alglucosidase alfa has been shown to be effective in improving survival and motor outcomes in infants with Pompe disease [11,12]. Age at disease symptom onset and stage of disease at treatment initiation have been noted as important factors in determining outcome; however, previous studies demonstrated that not all infants have favorable outcomes even when treatment is begun early. We have reported poor outcome in CRIM-negative patients [8], but no analysis of CRIM status across a large number of patients has been performed.

In the present analysis, CRIM-negative patients clearly show an attenuated response to enzyme in all outcome measures compared to CRIM-positive patients: significantly decreased survival, invasive ventilation-free survival, less improvement in cardiac response, and regression of motor milestones.

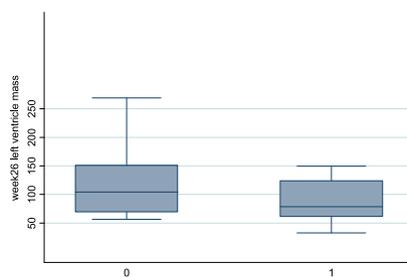
This analysis is necessarily limited by the small number of patients for whom robust, long-term clinical data are available. To control for confounding limitations of ERT in Pompe disease, we limited our patient group to those less than 6 months of age at onset of therapy [9]. In patients older than 6 months the muscle is likely in a more advanced stage of disease.

Poor outcome in the CRIM-negative patients could be due to those patients having generally more severe GAA gene mutations—that is, two deleterious mutations, which would be expected to result in complete absence of native enzyme and no band on Western blot. Interestingly, the CRIM-negative patients in this study were observed generally to have a period of improvement in the first 6 months of enzyme replacement followed by a period of decline. Given the coincident rise in antibody response, this sug-

Baseline LVM



Week 26 LVM



Week 52 LVM

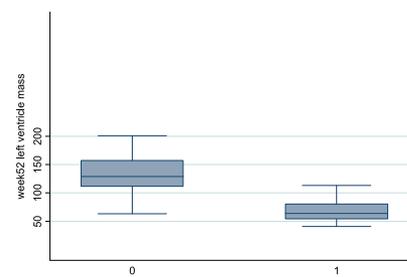


Fig. 3A. Left ventricular mass index (LVMI) at baseline and after 24 and 52 weeks of rhGAA treatment in CRIM-positive and CRIM-negative patients (labeled as 1 and 0 on X-axis, respectively).

Table 3

Antibody data and clinical status of infantile Pompe patients at database lock.

	Median (range) antibody titers					Last antibody titer	Week of last titer	Age at invasive ventilation (months)	Age at death (months)	Age at June 2006 database lock (months)
	Baseline	Week 24	Week 52	Week 64	Week 78					
CRIM-	negative	0 (0–100) n = 8	51,200 (12,800– 102,400) n = 7	153,600 (3,200– 1,638,400) n = 6	409,600 (204,800– 1,638,400) n = 4	12,800 (12,800) n = 1				
CRIM-	positive	0 (0–100) n = 19	600 (0–51,200) n = 20	200 (0–51,200) n = 19	300 (0– 51,200) n = 14	400 (0–25,600) n = 14				
Patient	Inhibition of uptake	Inhibition of activity	Seroconversion (week)	Peak antibody titer	Week of peak titer	Last antibody titer	Week of last titer	Age at invasive ventilation (months)	Age at death (months)	Age at June 2006 database lock (months)
<i>CRIM-negative</i>										
1	**	**	**	**	**	**	**	8.2	50.2	
2	**	**	**	**	**	**	**	8.3	44.8	
5	*	*	0	1:102,400	24	1:51,200	36	13.8	14.7	
8	*	*	0	1:204,800	64	1:204,800	64	11.3	23.3	
11	y	n	0	1:409,600	64	1:409,600	82	24.5	25.5	
20	y	y	4	1:1,638,400	52	1:1,638,400	64	18.5	32.0	
24	y	y	4	1:204,800	38	1:102,400	52	9.2		31
26	n	n	4	1:51,200	24	1:25,600	38	9.1		26
27	*	*	4	1:25,600	16	1:6,400	90		27.1	
28	n	n	0	1:409,600	64	1:409,600	64		18.0	***
31	**	**	**	**	**	**	**	25.3	51.4	
<i>CRIM-positive</i>										
3	**	**	**	**	**	**	**			83
4	n	n	4	1:51,200	38	1:25,600	78	15.0	41.0	
6	n	n	4	1:51,200	12	1:51,200	52			30
7	*	*	0	1:25,600	12	1:800	78			65
9	n	n	4	1:25,600	24	1:1,600	78			37
10	*	*	0	1:25,600	8	1:12,800	78		32.1	
12	n	n	4	1:12,800	12	1:200	78			41
13	n	n	4	1:12,800	16	1:3,200	80	24.4		30
14	n	n	4	1:6,400	20	1:200	52	19.4	19.8	
15	n	n	8	1:3,200	8	1:400	78			38
16	n	n	12	1:400	20	1:100	78			35
17	n	n	8	1:400	78	1:400	78			36
18	n	n	8	1:400	8	1:400	78			36
19	n	n	8	1:400	38	1:100	52			33
21	*	n	64	1:400	78	1:400	78			22
22	*	n	8	1:400	20	1:400	38			***
23	n	n	64	1:200	64	1:200	78	19.7		39
25	*	n	24	1:200	24	1:200	52			***
29	n	n	Never	0	0	0	78			33
30	n	n	Never	0	0	0	78			28
32	*	*	0	1:51,200	24	1:3,200	78			62

* Not done.

** Patients 1, 2, and 31 had different methods of antibody determination and are excluded from this analysis.

*** Patients were alive and younger than 18 months on June 2006; data were collected on these patients through age 18 months.

gests that the initial response to treatment with the exogenous enzyme is terminated by the increased antibody activity. Animal studies have suggested that antibody formation to rhGAA reduces the efficacy of ERT [21]. More recently, immune tolerance induction with an adeno-associated virus vector containing a liver-specific promoter in a Pompe knockout mouse model further supports the impact of antibodies in response to ERT [22]. We recently reported the successful induction of immune modulation in a CRIM-negative patient with Pompe disease using the anti-CD20 monoclonal antibody rituximab plus methotrexate and intravenous gamma globulin [23]. In this patient, ERT was started at 7 weeks of age, anti-rhGAA antibodies developed at 23 weeks, and immune modulation therapy was started at 25 weeks; at 24 months of age, the patient continued to be antibody free and to gain motor milestones on ERT.

The significant differences in clinical outcomes between the two CRIM groups in response to ERT were coincident with signifi-

cant differences in serotiter levels of antibodies against the enzyme. In the CRIM-negative group antibody titers were higher, seroconversion occurred earlier, and titers were sustained at higher levels. It appears that serotiter levels play a role in the clinical decline in these patients. Three of the 5 CRIM-positive patients who did poorly (i.e., died or were invasively ventilated) had peak titers of 12,800, 25,600, or 51,200; titers stayed elevated with no downward trend ever noted in two patients, while the third patient had a slight downward trend. Two of the five CRIM-positive patients with poor outcome had lower peak titers: one had a peak titer of 6400, which dropped to 200 at week 52, while one had zero titers until week 64 when titers increased to 200. Both of these patients had very poor AIMS scores at baseline with no apparent gains in motor function so it is likely that other factors were responsible for their poor outcomes.

Antibody interference has been described as a complicating factor in patients with other lysosomal storage disorders and with

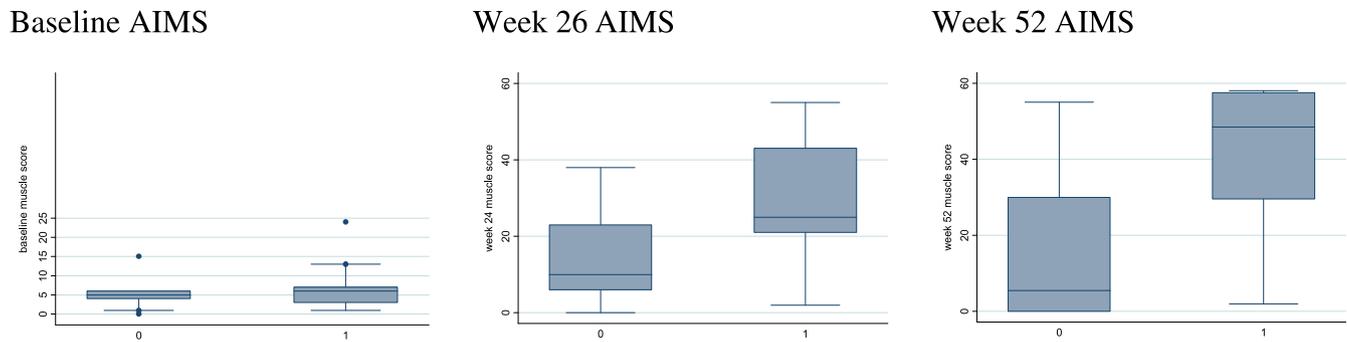


Fig. 3B. Alberta Infant Motor Scale (AIMS) scores at baseline and after 26 and 52 weeks of rhGAA treatment in CRIM-positive and CRIM-negative patients (Labeled as 1 and 0 on the x-axis, respectively).

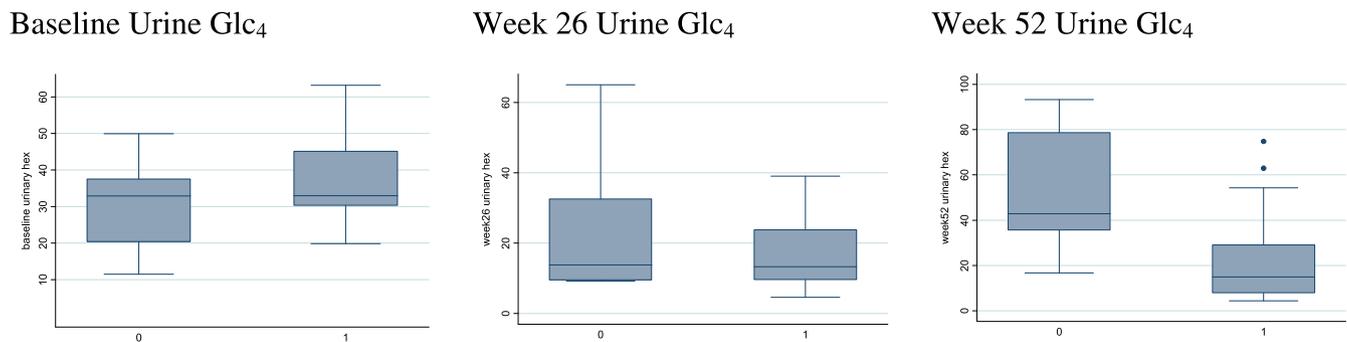


Fig. 3C. Urine Glc₄ levels at baseline and after 26 and 52 weeks of rhGAA treatment in CRIM-positive and CRIM-negative patients (Labeled as 1 and 0 on the x-axis, respectively).

other conditions for which a foreign protein is administered, such as hemophilia A and B [24–28]. Seroconversion rates in other LSDs vary; studies suggest that this variation is linked to the relative severity of gene mutations (and therefore potentially CRIM status) commonly seen in each disease [29,30]. More severe mutations are associated with CRIM negativity and a more marked immune response to the replacement enzyme, which appears to the immune system as a foreign protein. Resistance to factor replacement in patients with hemophilia A and B associated with the development of antibodies has been well described and is associated with mutations that produce absent or truncated factors [31]. The present analysis includes genotype data on 9 of 11 CRIM-negative patients; these patients all show severe mutations consistent with CRIM-negative status and a strong, sustained immune response to rhGAA.

Data from clinical trials [8,9,11] suggest that approximately 20% of all infantile Pompe cases are CRIM-negative, accounting for 6–7 cases of infantile Pompe disease in the US per year. Patients of African American descent who are homozygous for the p.Arg854X mutation account for at least half the CRIM-negative infantile patients identified in the US. Patients of Arabic Muslim descent who are homozygous for p.Lys114fsX32 are also identified to be CRIM-negative. Although only a small number of patients are expected to be CRIM-negative, investigation of CRIM status may assist in risk stratification of infantile Pompe patients and in selection of treatment interventions that have the potential to be successful in ablating antibodies, such as immune modulation, prior to start of or early in the course of ERT.

Resistance to therapeutic protein may be susceptible to modulation via tolerance-inducing protocols [32–34]. In many LSDs, although CRIM-positive patients generate antibody responses initially, such responses tolerate over a 1- to 2-year period on continued therapy [30,35]. Successful immunomodulation has been reported in animal models and in some patients with various factor or enzyme deficiencies for whom routine treatment was ineffective

[8,34,36–38]. For CRIM-negative patients with Pompe disease or severe Factor IX deficiency, reversing the antibody response once entrenched has generally not been successful, even with relatively strenuous pharmacologic approaches. Indeed, some patients developed nephrotic syndrome from immune complex-mediated nephritis on such therapies [39]. Additional tolerance-inducing strategies, such as the one described above [23], may include preventive tolerance induction at initiation of therapy or use of more novel B and T cell targeting agents to induce tolerance after the development of inhibitory antibodies. Immune tolerance via a gene therapy approach has also been shown to be efficacious in a GAA-KO mouse model of Pompe disease [22]. Clinical trials of immunomodulation must be conducted, as the outlook is otherwise dismal for CRIM-negative patients. Moreover, elucidating the immunologic mechanism of antibody development, especially with respect to the necessity for T cell help, may further define additional prophylactic or therapeutic targets for tolerance induction.

The potential benefit of enzyme replacement therapy and need for efficacious early intervention in Pompe disease has led to the development of dried blood spot GAA testing [40] and interest in incorporating GAA testing into newborn screening protocols. Blood-based assays of CRIM status are currently under development to rapidly determine CRIM status and drive treatment-related decisions (personal communication). Pompe disease is now becoming a treatable disease of infancy. Successful enzyme replacement therapies for other lysosomal storage disorders have become the standard of care, and similarly GAA replacement infusions are standard therapy for infantile Pompe disease, in conjunction with attentive supportive care for comorbidities [41]. CRIM-negative status currently means patients may have limited benefit from this expensive treatment, raising a difficult ethical dilemma: whether to withhold treatment from these patients. The treatment of CRIM-negative patients with ERT is therefore challenging; however, efforts are currently underway to develop tolerance-inducing therapies, which may offer a treatment path for such patients. This

study also challenges our understanding of the immune system's role in resistance to ERT and heightens the need for immunomodulation to improve ERT.

Disclosures

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rhGAA, in the form of Genzyme's product, Myozyme, has been approved by the US FDA and the European Union as therapy for Pompe disease. Duke University and inventors for the method of treatment and predecessors of the cell lines used to generate the enzyme (rhGAA) used in this clinical trial receive royalty payments pursuant to the University's Policy on Inventions, Patents and Technology Transfer.

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