

## B-Cell Depletion and Immunomodulation before Initiation of Enzyme Replacement Therapy Blocks the Immune Response to Acid Alpha-Glucosidase in Infantile-Onset Pompe Disease

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**Objective** To evaluate whether B-cell depletion before enzyme replacement therapy (ERT) initiation can block acid alpha-glucosidase (GAA) antibody responses and improve clinical outcomes.

**Study design** Six subjects with Pompe disease (including 4 cross-reacting immunologic material-negative infants) aged 2-8 months received rituximab and sirolimus or mycophenolate before ERT. Four subjects continued to receive sirolimus, rituximab every 12 weeks, and intravenous immunoglobulin monthly for the duration of ERT. Sirolimus trough levels, IgG, CD3, CD4, CD8, CD19, CD20, N-terminal pro-brain natriuretic peptide, creatine kinase, creatine kinase-MB, C-reactive protein, platelets, alkaline phosphatase, gamma-glutamyl transferase, aspartate aminotransferase, and alanine aminotransferase were measured regularly.

**Results** Immunomodulation achieved B-cell depletion without adverse effects. After 17-36 months of rituximab, sirolimus and ERT, all subjects lacked antibodies against GAA, 4 continued to gain motor milestones, yet 2 progressed to require invasive ventilation. The absence of infusion-associated reactions allowed the use of accelerated infusion rates.

**Conclusion** B-cell depletion and T-cell immunomodulation in infants naïve to ERT was accomplished safely and eliminated immune responses against GAA, thereby optimizing clinical outcome; however, this approach did not necessarily influence sustained independent ventilation. Importantly, study outcomes support the initiation of immunomodulation before starting ERT, because the study regimen allowed for prompt initiation of treatment. (*J Pediatr* 2013; ■: ■ - ■).

Immune responses to therapeutic proteins in patients with enzyme deficiencies secondary to severe mutations have been shown to limit long-term efficacy of enzyme replacement therapy (ERT), and have been particularly well described in Pompe disease.<sup>1</sup> Infantile-onset Pompe disease is the most severe phenotype of this recessive disorder and results in death from cardiorespiratory failure within 12-24 months in the absence of treatment. Milder forms of the disease presenting in children or adults are the result of less-deleterious mutations. Generally, acid alpha-glucosidase (GAA) activity <1% of wild-type level correlates with presentation in infancy, and 2%-20% GAA activity is seen in later-onset disease.<sup>1</sup> Approximately 25% of infants with <1% GAA activity have no detectable GAA protein by Western blot analysis and are considered cross-reactive immunologic material (CRIM)-negative.<sup>2</sup>

In CRIM-positive patients, the presence of residual GAA protein usually correlates with a lack of antibody (Ab) formation against GAA after initiation of ERT. In contrast, CRIM-negative patients lack tolerance to GAA protein and mount robust humoral immune responses to ERT, attenuating the efficacy to treatment. In a related adult study, 40% of administered recombinant alglucosidase alfa (Myozyme; Genzyme, Cambridge, Massachusetts) was captured by circulating anti-GAA Ab.<sup>3</sup> Thus, CRIM-negative patients receiving ERT have a poor prognosis and diminished survival unless some form of immunosuppression is administered.<sup>4</sup> Immunosuppressive drugs, including daily oral cyclophosphamide, methotrexate, omalizumab, and rituximab, have been used with some success, as has plasmapheresis.<sup>5,6</sup> In this 5-year study, we addressed the immunologic and clinical consequences of B-cell depletion and T-cell immunomodulation before initiation of ERT in infants with early-onset Pompe disease.

Ab	Antibody
CRIM	Cross-reactive immunologic material
ERT	Enzyme replacement therapy
GAA	Acid alpha-glucosidase
IV	Intravenous
IVIG	Intravenous immunoglobulin
LVMl	Left ventricular mass index

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## Methods

Five infants with the diagnosis of Pompe disease, GAA enzyme activity of <1%, and confirmed GAA mutations were enrolled into an observational study of Pompe disease at the University of Florida. Parents consented to pre-ERT immunosuppression between February 2007 and November 2010. An additional CRIM-positive patient with infantile-onset Pompe disease enrolled into the observational study who did not receive pre-ERT immunosuppression was included as a reference subject. The end date for analysis of results was March 15, 2012.

The study protocol was approved by the University of Florida Institutional Review Board. The patients' parents were informed about the current standard therapy, which is to initiate ERT as soon as the diagnosis of Pompe disease is confirmed by GAA activity assay. The option of receiving ERT without immunomodulation was made available as standard therapy if they chose not to begin immunomodulation. Stated risks of the immunomodulatory regimen included risk of infection, anaphylaxis, malignancy, and death. Written informed consent was obtained from the parents before initiation of immunosuppression.

Inclusion criteria for the study included diagnosis of Pompe disease before age 12 months; cardiac hypertrophy, as defined by a 2-dimensional left ventricular mass index (LVMI) >2 z-scores; GAA activity <1% in peripheral blood mononuclear cells or dried blood spots; absence of infection or complication that could be worsened by systemic immunosuppression; and no previous exposure to ERT.

After consent was obtained, all subjects received methylprednisolone (Pfizer, New York, New York) 10 mg/kg intravenously (IV), along with induction rituximab, given in 1 of 2 ways depending on the infant's clinical status and ability to tolerate IV fluids. Subjects A and E received two 750-mg/m<sup>2</sup> doses of rituximab, given 10-14 days apart. The other subjects received a loading dose of rituximab 375 mg/m<sup>2</sup> each week for 3 weeks, to decrease the fluid load with each dose. After the rituximab induction doses, each subject was started on daily immunosuppression with oral sirolimus (Wyeth, San Francisco, California) at a dose of 0.6-1 mg/m<sup>2</sup>/day, adjusted to maintain a trough serum sirolimus level of 3-7 ng/m. Subject A received mycophenolate (Roche, Genentech, San Francisco, California) 300 mg/m<sup>2</sup>/day at the start of the study instead of sirolimus.

After induction rituximab followed by oral immunosuppression, all patients were started on recombinant human  $\alpha$ -glucosidase (20 mg/kg IV every 7-10 days), initially infused over 6 hours. The ERT dosing interval was subsequently increased to every 10-14 days if clinical improvement was demonstrated, as evaluated by discontinuation of ventilatory assistance (invasive or noninvasive) and attainment of feeding goals, as well as discharge from the inpatient setting. ERT infusion rates were also increased stepwise over time to achieve a goal of 2-hour infusion periods as long as no infusion reactions were observed and no anti-GAA Ab were detected.

Once the induction doses of rituximab were completed, all subjects were started on monthly IV immunoglobulin (IVIG) infusions (Gamunex; Talecris Biotherapeutics, Research Triangle Park, North Carolina or Privigen; CSL Behring, King of Prussia, Pennsylvania) at a dose of 500-1000 mg/kg, adjusted to maintain a trough serum IgG level of 700-1000 mg/dL. IVIG was given to provide passive immunity, because subjects were not permitted to receive well-child vaccines other than the seasonal inactivated influenza vaccine for the duration of B-cell depletion. After initiation of ERT, maintenance rituximab at a dose of 375 mg/m<sup>2</sup> every 12 weeks was continued in 4 of the 5 subjects.

Archive-quality DNA was isolated from peripheral blood mononuclear cells using the Puregene Blood Core Kit (Qiagen, Venlo, The Netherlands) and then purified (MinElute; Qiagen) before polymerase chain reaction amplification and sequencing. Polymerase chain reaction-amplified products containing all 20 exons and the flanking intronic sequences of the GAA gene were generated using published flanking primers (Integrated DNA Technologies, Coralville, Iowa), sequenced at the University of Florida's Interdisciplinary Center for Biotechnology Research using an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, San Francisco, California), and then compared with published sequences (GenBank accession: NT\_024871.11) using Clone Manager Professional Suite, version 8 (Scientific and Educational Software, Cary, North Carolina).

CRIM status was evaluated at the Powell Gene Therapy Center (Gainesville, Florida) by Western blot analysis of skin fibroblasts. For this, 20  $\mu$ g of cell lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, run on 4%-12% gradient Tris-glycine gels, and transferred to nitrocellulose membranes. Blots were probed with a 1:10 000 dilution of a protein A-purified rabbit anti-GAA polyclonal Ab, allowing detection of unprocessed and processed forms of GAA, followed by incubation with goat anti-rabbit IRDye 800CW Ab at 1:20 000 dilution (LI-COR Biosciences, Lincoln, Nebraska). The membranes were scanned using infrared imaging (Odyssey; LI-COR Biosciences), and quantification of GAA was reported as integrated intensity, proportional to the amount of membrane-bound Ab (Figure 1; available at [www.jpeds.com](http://www.jpeds.com)).

Plasma anti-GAA Ab titers from patients and controls were measured by enzyme-linked immunosorbent assay at the Powell Gene Therapy Center. In brief, microtiter plates (Fisher, Pittsburgh, Pennsylvania) were coated with 5  $\mu$ g/mL recombinant human GAA protein overnight at 4°C, blocked with 10% fetal bovine serum for 2 hours at room temperature, and then washed with 0.05% Tween-20 in phosphate-buffered saline. Plasma samples diluted in blocking buffer were added to wells overnight at 4°C, washed with phosphate-buffered saline-Tween, and then incubated with a 1:10 000 dilution of secondary sheep anti-human IgG-horseradish peroxidase Ab (Amersham, Amersham, United Kingdom) at room temperature for 2 hours. Color development was performed using 3,3',5,5'-tetramethylbenzidine

(Invitrogen, Carlsbad, California), and reactions were stopped with 0.5 M sulfuric acid. Plates were read at a wavelength of 450 nm using a  $\mu$ Quant analyzer (Bio-Tek Instruments, Winooski, Vermont), and anti-GAA Ab titers were determined using standard curves generated with positive and negative controls.

Enumeration of B- and T-lymphocyte subpopulations in peripheral blood was done by flow cytometry using monoclonal Abs to CD19 or CD20 for B cells and monoclonal Abs to CD3, CD4, and CD8 for T cells in the University of Florida Shands Hospital Hematopathology Laboratory (Gainesville, Florida).

## Results

The 6 subjects with infantile Pompe disease (3 females and 3 males) were all from Florida and included 3 Hispanic infants, 2 Caucasian infants, and 1 African-American infant. Key features of the initial symptoms included difficulty feeding, failure to thrive, and cardiomegaly detected between age 3 weeks and 5 months. The 5 subjects receiving pre-ERT immunosuppression (subjects A, B, C, D, and E) were diagnosed with Pompe disease through measurement of GAA activity (<1% of normal between age 6 weeks and 7 months) and severe cardiac hypertrophy (LVMI >2 z-scores) (Table I). The reference subject was a CRIM-positive Caucasian infant (subject F), who received standard ERT without immunosuppression, presented with cardiac hypertrophy, and was diagnosed with infantile Pompe disease by enzyme assay of skin fibroblasts at age 8 months. Subject F was included in an earlier study<sup>7</sup> and was included here as a reference patient and for historical comparisons. Subject E presented with severe symptoms and received the same inductive immunosuppression regimen as the others, but was later determined to be CRIM-positive. CRIM-negative historical subjects serving as controls as all subjects anticipated to

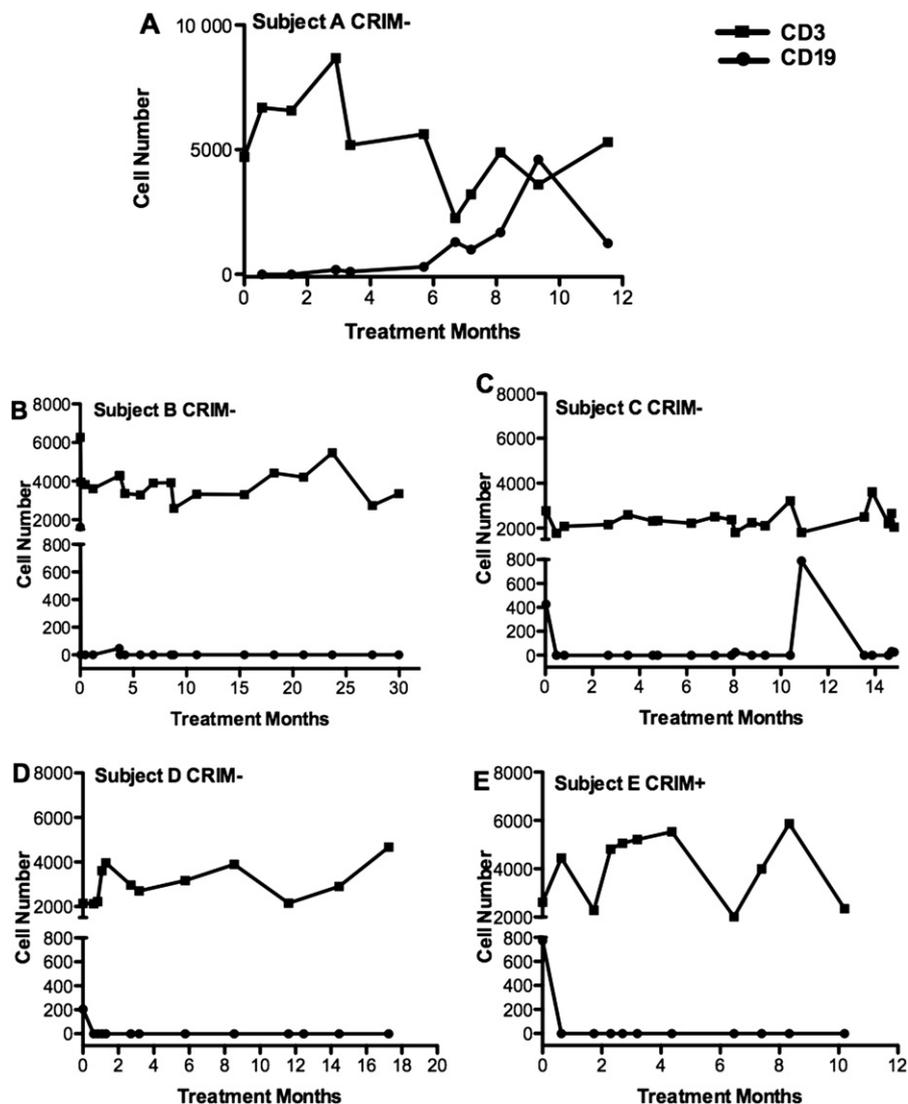
benefit from immune modulation and ERT were assigned to the study regimen. Given the established practice of initiating ERT on establishing a diagnosis of Pompe disease, we began immune modulation before GAA exposure to avoid any delay in initiation of ERT.

Patients with Pompe disease inherit 2 abnormal alleles of the GAA gene located on human chromosome 17. A comparison of the identified mutations in our patients with the database of the Pompe Center at Erasmus Medical Center Rotterdam, which includes mutation severity ratings, revealed that 2 CRIM-negative infants (subjects A and C) were homozygous for distinct mutations known to be correlated with severe and less severe disease phenotypes, respectively (Table I). Subject A was of African-American descent and was homozygous for the very severe Ex.18c2560C>T mutation, which is common in African-American patients. Subject C was homozygous for a missense mutation (Table I). Subject B was CRIM-negative and found to be a compound heterozygote for a very severe deletion and a previously unknown insertion that introduces a translational frameshift and a premature stop codon into the protein (Ex.12c.1705dupT) (Table I). Subject D was CRIM-negative, with 1 mutant allele, a putative severe nonsense mutation (Table I). Subject E was initially identified as CRIM-negative, but was later determined to be weakly CRIM-positive by Western blot analysis of cultured fibroblasts. The mutations in subject E included 1 very severe deletion and 1 potentially less-severe missense mutation (Table I). Subject F was CRIM-positive by Western blot analysis and was compound heterozygous for 2 less-severe GAA mutations (Table I).

At study enrollment and before induction of immunosuppression, all infants had normal B-cell and T-cell numbers and subset distributions for age except for subject C, who had persistent T-lymphocytopenia at study initiation that did not improve with interruption of daily sirolimus therapy (Figure 2). All subjects had normal serum

**Table I.** Characteristics of patients with infantile Pompe disease relative to CRIM status

	Subject A	Subject B	Subject C	Subject D	Subject E	Subject F
Sex	Female	Female	Male	Female	Male	Male
Age at diagnosis, months	4.5	7	1.5	5.5	2.5	6
Ethnicity	African American	Hispanic	Hispanic	Caucasian	Hispanic	Caucasian
GAA mutation allele 1	Ex.18 c.2560C>T	Ex. 9 c.1396delG	Ex.5 c.925G>A	Ex.10 c.1548G>A	Ex. 14 c.1933G>A	Ex.3 c.655G>A
Nucleotide position	p.Arg854X	p.Val466PhefsX11	p.Gly309Arg	p.Try516X	p.Asp645Asn	p.Gly219Arg
Type of mutation	Nonsense	Deletion, frameshift, early stop	Missense	Nonsense	Missense	Missense
GAA mutation allele 2	Ex. 18 c.2560C>T	Ex. 12 c.1705dupT	Ex.5 c.925G>A	Not found	Ex. 18 c.2501_2502del	Ex.12 c.1735G>A
Nucleotide position	p.Arg854X	p. Tyr569LeufsX67	p.Gly309Arg	Not found	p.Thr834ArgX49	p.Glu579Lys
Type of mutation	Nonsense	Insertion, frameshift, early stop; new mutation	Missense	Not found	Deletion	Missense
CRIM status	Negative	Negative	Negative	Negative	Positive	Positive
GAA activity	4 nmol/h/mg (white blood cells); <1%	1.3 pmol/punch/h (dried blood spot)	1.2 nmol/mg protein/h (leukocytes); <1%	7-9 pmol/h/spot (dried blood spot); <1%	9 pmol/h/spot (dried blood spot); <1%	1.3 nmol/h/mg (skin fibroblasts); <1%
Start of immune suppression, month	8	8	2	5.5	3	None
Start of ERT, month	8	8	2.75	6	3	~9
Age at end of study, months	19	44	33	30	19	116



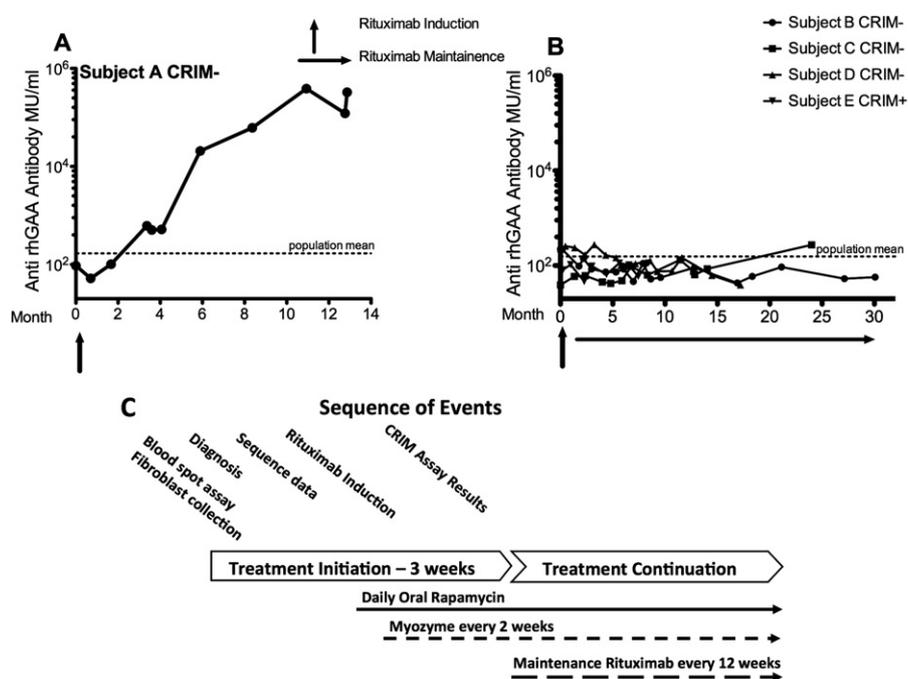
**Figure 2.** Enumeration of peripheral blood CD3-positive T cells and CD19/CD20-positive B cells by flow cytometry in subjects with infantile Pompe disease on immunosuppression over time.

immunoglobulin levels (IgG, IgA, and IgM) and lymphocyte proliferative responses to pokeweed mitogen, phytohemagglutinin, and concanavalin A (data not shown). Induction rituximab was given either as 2 doses of 750 mg/m<sup>2</sup> 10-14 days apart, or at 375 mg/m<sup>2</sup>/dose weekly for 3 weeks in subjects with suspected sensitivity to larger fluid volume infusions. All subjects were effectively depleted of all circulating CD19/CD20-positive B cells by flow cytometry. B-cell repopulation was not observed in any subjects who continued receiving maintenance rituximab every 12 weeks while on ERT.

Subject A received daily mycophenolate 300 mg/m<sup>2</sup>, and subjects B, C, D, and E received daily sirolimus at doses adjusted to maintain a serum trough level of 3-7 ng/mL. All infants tolerated immunosuppression well, and all but subject C had T-cell numbers and subset distributions within normal limits for age for the duration of the study.

During the time period in which subjects had undetectable peripheral CD19/CD20-positive B cells after rituximab therapy, total serum immunoglobulins (IgA, IgM, and IgG) were low and anti-GAA-specific Ab was undetectable (Figure 3 and data not shown). Subject A received only induction rituximab, and without maintenance rituximab infusions, B cells were detected after 4 months. The subject continued to receive mycophenolate during this period, and detection of anti-GAA Ab was associated with the return of CD19/CD20-positive B cells. Three months after B-cell recovery was noted, her serum immunoglobulin levels normalized (data not shown), and she received no further IVIG. The return of B-cell function was associated with rapid production of high-titer Ab against GAA, subsequent clinical deterioration, and death 5 months later.

Subjects B, C, D, and E continued to receive ongoing B-cell depletion with every-12-week rituximab infusions and daily



**Figure 3.** Anti-GAA Ab titers in subjects with infantile Pompe disease receiving pre-ERT immunomodulation. **A**, CRIM-negative subject A treated with induction rituximab only, followed by ERT and daily mycophenolate. **B**, CRIM-negative subjects B, C, D, and E treated with induction and maintenance rituximab, daily sirolimus, and ERT. Rituximab, sirolimus and Myozyme were discontinued for subject B at ~13 months after initiation of treatment. **C**, Sequence of treatment events for immunomodulation and ERT.

sirolimus which prevented anti-GAA Ab formation. In subject C, discontinuation of rituximab after 10 months of ERT resulted in reemergence of peripheral blood B cells within 4 months despite daily sirolimus; however, anti-GAA Ab was not detected at the time of B-cell recovery. At 16 months after discontinuation of rituximab and 14 months after discontinuation of sirolimus, his serum anti-GAA Ab titer remained undetectable (Figure 3). Subject C was unique in having the least favorable clinical response of all the study subjects.

No anti-GAA Ab formation was detected in subjects B, C, D, and E. Accelerated infusion schedules were achieved in all of these subjects, as well as in subject F, who had remained GAA Ab-negative because he was strongly CRIM-positive. ERT was administered at the standard dose of 20 mg/kg every 7-14 days; however, the infusion rate was accelerated to deliver the total GAA dose over 2 hours. This was accomplished by giving 10% of the total dose within the first 30 minutes as an initial test dose, followed by the remaining 90% over the subsequent 90 minutes. No infusion-associated reactions were observed with this schedule. Of note, more frequent infusions of ERT were achieved without adverse events. Subject F, who did not produce anti-GAA Ab, also received ERT 20 mg/kg every 14 days over 2 hours without complications. This accelerated infusion schedule was pursued to improve the theoretical peak concentration of GAA during infusion and to maximize delivery by receptor-mediated uptake.

No subject developed opportunistic or serious bacterial infections that could be directly attributed to immunomodulation. Subject B experienced chronic gram-negative urinary tract infections related to uncorrected grade III vesicourethral reflux despite prophylactic antibiotics, as well as an episode of *Clostridium difficile* colitis. Subject C required bilevel positive airway pressure therapy and subsequent tracheostomy and chronic invasive ventilation at age 10 months after 4 months of ERT. He had multiple viral respiratory illnesses that did not resolve or improve with interruption of daily sirolimus therapy for 2-8 weeks at a time over a 6-month course of immunosuppression. After tracheostomy, subject C restarted daily sirolimus and no longer experienced upper respiratory infections, although a decreased peripheral blood CD3 cell count persisted. However, rituximab was discontinued after 10 months, and sirolimus was discontinued 2 months later after a serious adverse event related to complications of mechanical ventilation. The subject suffered respiratory arrest and neurologic injury after mechanical failure of assisted ventilation. He continued to receive IVIG and ERT until his transfer to a hospice facility, where the decision was made to discontinue all treatments. He remained alive on assisted ventilation 16 months later at conclusion of the study. None of the other subjects experienced any adverse events.

In subject A, echocardiography showed no significant change in LVMI after 10 months of ERT, although analysis revealed a significantly decreased level of serum N-terminal probrain natriuretic peptide, a biomarker for left ventricular

**Table II.** Improved cardiac markers and increased ventilator-free survival in subjects with early onset Pompe disease receiving immunomodulation

	Subject A	Subject B	Subject C	Subject D	Subject E	Subject F
Anti-recombinant human GAA titer at end of study	Very high	None	None	None	None	None
Duration of ERT, months	10	36	31	34	16	107
LVMI before ERT, g/m <sup>2</sup>	191.22	199.97	93.49	61.05	61.72	35.57
LVMI at end of study, g/m <sup>2</sup>	162.42	118.15	44.11	69.05	61.72	35.57
NT-proBNP at beginning of study	22 328	9110	8987	>54 000	34 975	<50
NT-proBNP at end of study	292	3578	<50	<50	160	-
Chronic ventilator support during study (age at ventilation, months)	No (died)	No*	Yes (17)	No	No	Yes (47)

NT-proBNP, N-terminal probrain natriuretic peptide.

\*After the study period, subject B developed the need for ventilator support at age 43 months.

function (Table II). In contrast, subjects B, C, D, and E, who received rituximab every 12 weeks while on ERT, showed reductions in LVMI (Table II). The rate of change in LVMI and degree of improvement were not significantly different than reported previously for infants with Pompe disease receiving ERT.

Subject A, who received only induction rituximab, experienced progressive cardiopulmonary failure, and the family elected not to pursue invasive ventilation. Subject B was managed without a tracheostomy until nearly age 4 years when she developed slowly progressive increased work of breathing, especially at night. She was managed for a brief period with bilevel positive airway pressure therapy, but eventually required invasive ventilation after the end of the data collection period for this study. Subject C remained marginally compensated at baseline and also required noninvasive ventilatory assistance and intensive care during any episodes of illness or low-grade fever, possibly related to his baseline T lymphocytopenia. Ultimately, he required tracheostomy and invasive ventilation at age 10 months, despite the absence of anti-GAA Ab. Subject D required assisted ventilation at the time of diagnosis of infantile Pompe disease for 2 months, but was subsequently ventilator-independent through 22 months of ERT. In contrast, subject E never required invasive ventilation and had no evidence of respiratory deterioration at the end of the study after 17 months of ERT. Subject F, who did not receive immunosuppression, developed progressive muscle weakness and has required assisted ventilation for the last 8 years.

Subject B developed severe mitral regurgitation as a result of cardiac dilation, which contributed to congestive heart failure and the subsequent need for tracheostomy and invasive ventilatory support during sleep after 36 months of ERT. Subjects B, C, and F developed significant sleep disturbance detected by polysomnography at the completion of the study. These findings further underscore the difficulties faced when managing ventilatory deficits in this patient population.

Subjects B, D, and E continued to make developmental strides and gain muscle strength globally. All subjects had persistent markedly elevated creatine kinase and creatine kinase-MB levels at the end of the study (data not shown). Aided by intensive physical rehabilitation, these infants were able to achieve independent sitting at the end of the

study, and subjects B and D were able to pull to standing and bear weight; however, no subject achieved independent ambulation.

All subjects demonstrated mild cognitive delay, more in speech than in receptive language, but to a lesser degree than suggested by registry data<sup>8</sup> (data not shown). In subjects B and D, brain magnetic resonance imaging demonstrated abnormal myelination without atrophy after 32 months and 12 months of ERT, respectively. Subject B had mild sensorineural hearing loss bilaterally detected by ear-specific visual reinforcement audiometry after 32 months of ERT. Brain magnetic resonance imaging of subject E was normal after 11 months of ERT. All subjects required a gastrostomy tube to maintain adequate caloric intake.

## Discussion

Immune response to protein replacement therapy has long been recognized as a limitation of the use of recombinant proteins, especially in recessive conditions such as Pompe disease. A previous study of ERT in 3 infants with Pompe disease documented the clinical decline of 2 infants who developed high and sustained Ab titers to GAA.<sup>9</sup> In the pivotal study leading to the approval of alglucosidase alfa, high rates of infusion-associated reactions (95%) and seroconversion (88%) prompted the consideration of immune modulation in our subject A, who was started on rituximab and mycophenolate in February 2007, immediately after the commercial release of Myozyme.

Although initial studies focused on CRIM-negative patients with null GAA mutations and no residual GAA protein, recently it has become clear that some patients who are characterized as CRIM-positive by current assay methods will also develop Ab to GAA.<sup>3</sup> Immunomodulation of such high-risk patients using rituximab, as well as the anti-IgE Ab omalizumab, has been used to control anti-GAA Ab production during ERT<sup>10,11</sup>; however, elimination of existing Ab-producing plasma cells remains a significant challenge, given that these cells do not express CD20 and are resistant to rituximab, a humanized anti-CD20 Ab that depletes by targeting B-cell surface CD20 antigen.<sup>12</sup> The side effects of increasingly toxic immunotherapy to inhibit preestablished anti-GAA immune responses may in fact outweigh any gains achieved by ERT.

Our findings indicate that immunomodulation of high-risk infants with Pompe disease (both CRIM-negative and CRIM-positive) is safe, effectively controls anti-GAA responses, and eliminates the need for more intensive immunosuppression after ERT initiation with the development of anti-GAA Ab and infusion-associated reactions. In this study, we used sirolimus, an inhibitor of the mammalian target of rapamycin pathway, rather than cytotoxic drugs or signal blockers to modulate T-cell responses to recombinant GAA. We hypothesized that sirolimus was advantageous considering previously reported evidence that sirolimus selectively promotes the survival and expansion of regulatory T cells while allowing for programmed cell death of activated effector T lymphocytes.<sup>13-16</sup> Clinical studies support improved immune regulation using sirolimus<sup>17</sup>; thus, we believe the optimal immune modulatory regimen in this patients population benefits from the properties of both rituximab and sirolimus.

Pretreatment with only an induction dose of rituximab and maintenance mycophenolate proved insufficient to control anti-GAA Ab formation in subject A. B-cell recovery and detection of low-level anti-GAA Ab after 4 months of ERT were the initial clues that subject A had developed a resurgent immune response with considerably increased anti-GAA Ab after 7 months of ERT. However, rituximab pretreatment followed by sirolimus and ERT within 2 weeks of the initial rituximab dosing and maintenance rituximab every 12 weeks proved to be a straightforward and successful protocol for avoiding anti-GAA immune responses. This protocol successfully depleted B cells in the 4 subjects receiving this immunomodulatory regimen. None of these subjects exhibited an immune response to GAA, including absence of anti-GAA Ab, for the duration of the study (17-36 months).

Passive immunity was provided with monthly IVIG infusions, and no subjects experienced bacterial or viral infections other than recurrent urinary tract infections in subject B with uncorrected vesicourethral reflux and the chronic viral respiratory illnesses in subject C until tracheostomy placement. The study cohort, which included 4 CRIM-negative infants, had the same rate of respiratory failure as subjects in the previous pivotal ERT studies that were subsequently evaluated in the Pompe disease registry. Studies of additional patients are needed to validate this finding.<sup>7,8</sup> All subjects except subject C received ERT at home, and the absence of anti-GAA Ab allowed for accelerated infusion rates with no infusion-associated reactions (a total of 433 infusions in the cohort). Subject E received immunomodulation owing to the severity of his phenotype, although he was subsequently found to be weakly CRIM-positive based on Western blot analysis of skin fibroblasts. A defined CRIM assay is needed to differentiate weakly CRIM-positive patients, who are at high risk for developing anti-GAA immune responses, from patients who are nonresponsive to alglucosidase alfa. Subject F did not develop an ERT infusion reaction or related immune response, highlighting the wide range of reactions observed among patients with early-onset Pompe disease and the difficulty in predicting the risk of subsequent anti-

GAA immune responses without an early and detailed CRIM assay. The decision to initiate immune modulation was based on the age of onset and severity of symptoms. In our opinion, determining the need for continuing immune modulation requires an individualized approach based on overall clinical condition and response to treatment. Further experience in this limited patient population will certainly help establish more definitive guidelines for management of this challenging disease.

In addition to preventing Ab formation against GAA, our regimen produced a favorable reduction in surrogate markers of heart failure and expected reductions in LVMI. The density of mannose-6-phosphate receptor and blood flow in the heart favor therapeutic protein delivery, which may explain a more consistent response to ERT in the heart. Importantly, despite maximal delivery of alglucosidase alfa with early and weekly ERT dosing (up to 40 mg/kg), there was no further augmentation of effectiveness in cardiac mass reduction over that reported in the first pivotal alglucosidase alfa study.<sup>7</sup> We followed the same dosing regimen as in that initial study, in which the GAA dose was increased in subjects with declining clinical condition. This finding suggests that the limitation of current therapies is not related solely to Ab-mediated changes in the biodistribution of GAA. Other possible factors include the limited phosphorylation on mannose residues of the currently available product and inefficient trafficking of GAA from the cell surface *in vivo* compared with that reported in previous *in vitro* studies. A direct impact on glycogen synthase may be another potential benefit of the use of sirolimus in this setting, given the recent findings in an animal model of Pompe disease.<sup>18</sup> The high-dose cohort in the initial pivotal ERT study had a higher rate of infusion-associated reactions, whereas our subjects in the present study had no infusion-associated reactions, reinforcing the relationship between anti-GAA Ab and infusion-associated reactions.

Another critical outcome measure for studies in early-onset Pompe disease is the management of respiratory insufficiency. Early after clinical presentation, severe cardiac hypertrophy leads to reduction in intrathoracic volume that results in a loss of left lung volume. Our subjects were all initially managed with high flow through a nasal cannula using room air to avoid mechanical ventilation. This strategy might have facilitated the avoidance of assisted ventilation during the first 4 months of ERT in subjects A, B, C, and E. During the follow-up period, subjects were closely monitored for evidence of ventilatory insufficiency, and subjects A, B, and C developed progressive respiratory insufficiency to a variable degree, especially when challenged with intercurrent, usually mild viral illnesses that led to increased ventilatory demand.

The proportion of children in our small cohort who required mechanical ventilation is the same as that reported in pivotal alglucosidase alfa studies<sup>7,19</sup> and in registry data.<sup>19,20</sup> These findings are not unexpected, given the recent findings from preclinical studies that confirm a neural component of respiratory dysfunction in the mouse model of

Pompe disease.<sup>21</sup> ERT is unable to cross the blood–brain barrier and has no influence on the lower motor neuron dysfunction that contributes to respiratory failure.<sup>21–23</sup>

Evaluation of developmental outcomes in this patient cohort demonstrates improvement over that reported in previous studies,<sup>7,19</sup> particularly in the acquisition of motor skills, although all subjects demonstrated significant delays compared with unaffected age-matched controls. All infants were able to sit independently, and 2 were able to bear weight on their lower extremities, with no evidence of regression in motor skills among those who were gaining function. In contrast, CRIM-negative subjects in the initial pivotal studies did not survive past age 36 months. These findings emphasize the importance of an individualized approach to the management of early onset Pompe disease.

Prevention of Ab formation against GAA by B-cell depletion and T-cell immunomodulation was safe, eliminated serious infusion reactions, and facilitated some improvement in clinical outcome. However, lack of anti-GAA neither significantly augmented recovery of nerve and muscle function nor resolved residual cardiac glycogen storage, confirming that reduced efficacy of ERT is not due solely to anti-GAA effects. Importantly, immunomodulation should be initiated before ERT in all cases of early onset Pompe disease. ■

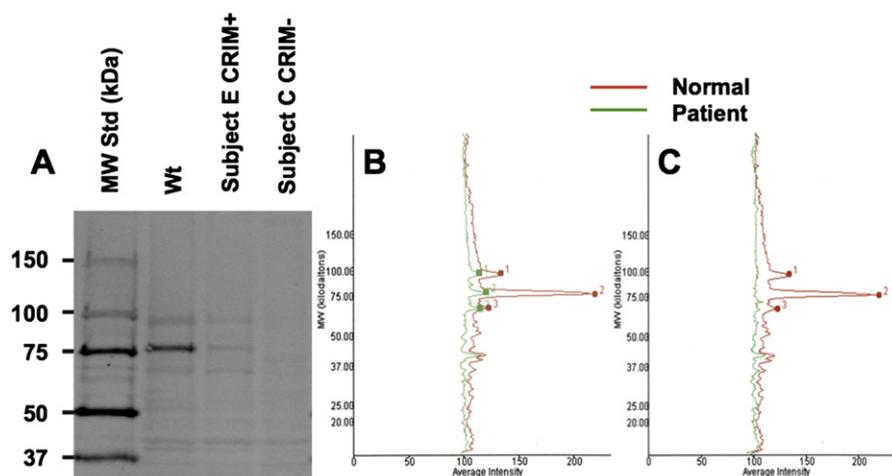
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**Figure 1.** **A**, Representative CRIM Western blots for normal control, CRIM-positive (subject E), and CRIM-negative (subject C) patients with Pompe disease. **B**, Integrated intensity of CRIM-positive subject E vs normal control. **C**, Integrated intensity of CRIM-negative subject C vs normal control.