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CHINESE HAMSTER OVARY CELL-DERIVED RECOMBINANT HUMAN ACID α -GLUCOSIDASE IN INFANTILE-ONSET POMPE DISEASE

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Abstract

Objective—To conduct an open-label, multinational, multicenter study examining the safety and efficacy of recombinant human acid α -glucosidase (rhGAA) in treatment of infantile-onset Pompe disease.

Study design—We enrolled 8 infant patients who had Pompe disease with GAA activity <1% of normal, cardiomyopathy, and hypotonia. In the 52-week initial phase, rhGAA was infused intravenously at 10 mg/kg weekly; an extension phase continued survivors' treatment with 10 to 20 mg/kg of rhGAA weekly or 20 mg/kg every 2 weeks for as long as 153 weeks. Safety measurements included adverse events, laboratory tests, and anti-rhGAA antibody titers. Efficacy evaluations included survival, ventilator use, echo-cardiograms, growth, and motor and cognitive function.

Result—After 52 weeks of treatment, 6 of 8 patients were alive, and 5 patients were free of invasive ventilator support. Clinical improvements included ameliorated cardiomyopathy and improved growth and cognition. Five patients acquired new motor milestones; 3 patients walked independently. Four patients died after the initial study phase; the median age at death or treatment withdrawal for all patients was 21.7 months, significantly later than expected for patients who were not treated. Treatment was safe and well tolerated; no death was drug-related.

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Conflict of Interest: P.S.K., A.A., and Y.T.C. have received research/grant support from Genzyme Corporation. P.S.K. and M.N. are members of the Pompe Disease Advisory Board for Genzyme Corporation. Y.T.C. has served as a consultant for Genzyme Corporation. If therapy for Pompe disease proves successful commercially, Duke University and the inventors of the cell line used to generate the enzyme used in this clinical trial (CHO-1 in this manuscript) may benefit financially pursuant to the Duke University's Policy on Inventions, Patents, and Technology Transfer.

Conclusion—rhGAA improved ventilator-free survival, cardiomyopathy, growth, and motor function in patients with infantile-onset Pompe disease compared with outcomes expected for patients without treatment.

Pompe disease (also known as glycogen storage disease type II, acid maltase deficiency, or glycogenosis type II) is a rare autosomal recessive metabolic muscle disease. Deficiency in acid α -glucosidase (GAA) results in lysosomal accumulation of glycogen in many body tissues and ultimately leads to multisystemic pathology.

Historically, Pompe disease has been classified into different subtypes on the basis of the age at symptom onset, extent of organ involvement, and rate of progression to death.¹ The clinical spectrum ranges from a rapidly progressive form (infantile-onset) to a more slowly progressive form (late-onset), with considerable variability and overlap in these extremes.^{2–4} The combined incidence of all forms of Pompe disease is approximately 1:40,000.^{5,6}

Patients with infantile-onset Pompe disease typically present before 12 months of age with progressive, hypertrophic cardiomyopathy that may obstruct left ventricular outflow; profound muscle weakness and hypotonia; non-attainment or loss of motor milestones; difficulty feeding; and failure to thrive. These patients have a dramatically shortened life span. In patients who are not treated, the median age of death ranges from 6.0 to 8.7 months.^{4,7} In the most rapidly progressive form, also termed “classic” infantile Pompe disease, the mortality rate is as high as 92% to 95% in the first year of life.⁷ In an historical cohort of patients manifesting Pompe disease in the first year of life, irrespective of phenotype, 74% died by 1 year of age, 91% by 2 years of age, and 93% by 3 years of age.⁷ Death generally results from cardiac and respiratory failure.^{1,3,7}

No approved specific treatment for Pompe disease currently exists. However, recombinant human GAA (rhGAA) has shown physiological activity both in animal disease models and in early clinical trials.^{8–15} In 3 pilot studies in severely affected infants, rhGAA (purified from transgenic rabbit milk^{11–14} or from Chinese hamster ovary [CHO] cell cultures⁸) markedly ameliorated cardiomyopathy and prolonged all patients’ survival beyond 1 year. One of 6 patients given rhGAA from rabbit milk (a preparation that is no longer available) and 1 of 3 patients given CHO cell-derived rhGAA walked independently and remained ventilator-free. The remaining 7 patients from these 3 studies showed lesser degrees of motor improvement and eventually required ventilation. As of January 2006, 3 of the 9 patients in these pilot studies had died and 6 remained alive (unpublished data). The 3 patients who died ranged in age from 1.2 to 4.3 years at the time of death, and the 6 patients who were living ranged in age from 3.7 to 6.5 years as of January 2006 (unpublished data).

Some patients with Pompe disease have a small amount of natural, but inactive, GAA enzyme. This material is called cross-reacting immunologic material (CRIM) because it, like rhGAA, is recognized by anti-GAA antibodies. In the Amalfitano et al CHO-cell rhGAA study,⁸ patients who did not become ambulatory lacked CRIM (as determined by means of Western blotting) and produced high anti-rhGAA antibody titers.⁸ In the Van den Hout et al study,¹¹ the patient with the best motor response to rhGAA from transgenic rabbit milk was also CRIM-positive, although these authors did not find a correlation between CRIM status and antibody formation and did not consider that antibodies had an effect on clinical outcomes.

To confirm the promising preliminary results of these studies in a larger group of patients, investigators in the United States and Europe conducted this phase II, open-label, multicenter, multinational trial of enzyme replacement therapy (ERT) with CHO-derived rhGAA in 8 patients with infantile-onset Pompe disease. Results from the initial 52 weeks of therapy and an extension study are presented here, along with the current status of surviving patients.

METHODS

This trial was initially conducted at centers in the United States and Europe and was approved by their institutional review boards and ethics committees. Written informed consent was obtained from parents or guardians. Eight patients with skin fibroblast GAA activity <1% of the normal mean (assayed against 4-methylumbelliferyl- α -D-glucopyranoside), cardiomegaly (cardiothoracic ratio >0.5 by means of chest radiography), and left ventricular hypertrophy (left ventricular mass index [LVMI] ≥ 65 g/m², ≥ 2 SD higher than the age-appropriate normal mean¹⁶) were enrolled. Exclusion criteria included respiratory insufficiency (O₂ saturation in room air <90%, arterial pCO₂ >50 mm Hg, or any ventilator use), signs and symptoms of cardiac failure and a cardiac ejection fraction <40%, or a major congenital abnormality or significant organic disease (unrelated to Pompe disease and likely to decrease survival rate). Patients still living after the 52-week initial phase were enrolled into an extension protocol.

Study Methods and Treatments administered

Two preparations (CHO-1 and CHO-2) of the investigational product, rhGAA, were purified from 2 different CHO cell lines transfected with the cDNA for human GAA. The protein sequences of the rhGAAs produced from each preparation are identical to each other and to a commonly occurring form of human GAA, with a calculated protein mass of 99.4 kDa. The recombinant proteins also contain 7 asparagine-linked glycosylation sites and 13 cysteine residues, 12 of which are involved in disulfide linkages. Both enzymes have similar specific activities toward a synthetic substrate and were prepared as 110-kD precursor proteins in a frozen liquid or lyophilized form.

All patients underwent baseline assessments within the 7 days before their first infusion of rhGAA. During the initial 52-week phase, all patients started receiving weekly intravenous infusions of CHO-1 at a dose of 10 mg/kg. In an attempt to improve overall clinical response, the doses were increased for patients H, C, and F. The dose for patient H was increased to 20 mg/kg weekly after week 43 of the initial phase and maintained at that level for 26 further doses. Patient C received 2 doses at 20 mg/kg, starting after week 90, and patient F received 10 doses, starting after week 70. All doses of rhGAA were administered in 3-hour infusions (2 mg/kg for 30 minutes, and the remaining dose for 2.5 hours). An independent safety monitoring board reviewed safety data. Exposure to CHO-1 ranged from 17 to 100 weeks. When a more robust manufacturing process was developed, patients who survived (patients A, C, E, and F) were transitioned to the second rhGAA preparation (CHO-2). These 4 patients received CHO-2 for periods ranging from 17 to 54 weeks. Total exposure to rhGAA, including the extension phase and both preparations, ranged from 17 to 153 weeks. At the time this study was initiated, results from preclinical studies in animal models^{9,10} and results from previous clinical trials^{8,11,14} suggested that a dose higher than that required for ERT for other lysosomal storage disorders was necessary to have a clinical effect in Pompe disease.

Study Assessments

Safety evaluations included assessment of adverse events (AE); routine physical examinations; vital signs; routine blood and urine tests, including CBC with differential, blood chemistry panels, and urine analysis; and anti-rhGAA titers. AEs are defined as clinical events occurring after initiation of rhGAA treatment; infusion-associated reactions (IARs) are AEs occurring during or immediately after the rhGAA infusion, which may be responses to recombinant protein.^{17–20} Hearing was evaluated by means of oto-acoustic emission (OAE) or brainstem auditory evoked response (BAER) testing.

Efficacy was assessed by measuring survival, time to ventilator dependence (continuous need for invasive ventilation), an echocardiographic index of cardiomyopathy (LVMI), motor

development (as measured by using the Alberta Infant Motor Scale [AIMS]²¹), and growth (weight, length, and head circumference). Cognitive function was monitored by using the Modified Bayley Scales of Infant Development, second edition, BSID-II.²²

Muscle GAA activity and histomorphometric analysis of muscle glycogen

GAA activity was analyzed in quadriceps muscle biopsies obtained before rhGAA treatment and 12 and 52 weeks after initiation of rhGAA treatment; samples measuring approximately $0.5 \times 0.5 \times 3$ cm were taken from alternating quadriceps muscles at each point with general, regional, or local anesthesia. Week 12 and 52 biopsies were taken 7 days after the last rhGAA infusion. For the GAA assay, frozen muscle biopsies were homogenized, sonicated, and centrifuged; GAA activity in the $2000 \times g$ supernatant was assayed with 4-methylumbelliferyl- α -D-glucoside (4-MUG) and quantitated against an 8-point 4-MU standard curve prepared in 0.2 mol/L sodium acetate, 0.4 mol/L KCl, 0.01% pH 4.3 dilution buffer. The curve ranged from 5.0 to 0.005 nmol/well 4-MU. The assay was stopped by the addition of 0.5 M carbonate/bicarbonate, pH 10.65, and fluorescence was measured at 355 nm/466 nm. GAA activity was reported as nmol/hour/mg of total protein. The intraassay-precision between replicates was <10% coefficient of variation and overall inter-assay precision for 4 assays performed by 3 analysts ranged from 5.3% to 16.5% coefficient of variation.

Muscle specimens were also analyzed for glycogen content by means of histomorphometric analysis. Skeletal muscle biopsies were prepared and analyzed with the method of Lynch et al.,²³ which generates high-contrast periodic acid-Schiff-stained images of well-preserved glycogen granules. Digital high-resolution light micrographs at a standard pixel density were analyzed with MetaMorph image processing and analysis software (version 4.6; Universal Imaging Corporation) to determine total accumulated glycogen area as a percentage of total stained section area in a representative microscope field. One representative field was read per slide; the readers were not blinded to when the sample was collected. The mean plus or minus SD of glycogen content was determined for as many as 10 slides per patient time-point.

CRIM Status

CRIM status was determined as described by Klinge et al.¹⁴ In brief, patient fibroblasts were cultured, harvested, and disrupted by using sonication in the presence of protease inhibitors. The resultant cell lysate was subjected to Western blotting analysis with a pool of monoclonal antibodies specific for GAA. These monoclonal antibodies were generated by using purified placental-derived GAA and have been shown to recognize both native and recombinant GAA. Control samples included normal human foreskin fibroblast lysates (native GAA), recombinant GAA, and CRIM(-), and CRIM(+) cell lysates.

Anti-rhGAA Antibodies

We developed an enzyme-linked immunosorbent assay for anti-rhGAA antibodies. Dilutions of patient serum were incubated in wells of rhGAA-coated microtiter plates; bound antibodies were detected by using a horseradish peroxidase-goat anti-human immunoglobulin (Ig) G Fc-specific antibody and 3,3',5,5'-tetramethylbenzidine substrate. Antibody levels were expressed as titers; their specificity was confirmed by means of radioimmunoprecipitation.

GAA Gene Mutation Analysis

DNA was extracted from whole blood by using the PureGene DNA Isolation Kit (Gentra, Minneapolis, Minn), amplified with GAA gene-specific oligonucleotide primers,²⁴ and then analyzed on a Transgenomic Wave apparatus, which detects DNA polymorphisms by strand separation, mismatched reannealing, and high-performance liquid chromatography of the resulting heteroduplexes. Patient or parent samples were run alongside known normal samples;

differences in the chromatograms indicated potential allelic variations. Amplified products were sequenced on an Applied Biosystems 377 or 3100 automated DNA sequencer with ABI BigDye v3 reagents, then compared with a consensus sequence (RefSeq #NM_000152) for the human GAA cDNA compiled from normal individuals of Caucasian, Asian, African-American, and Ashkenazi Jewish populations to determine whether patient sequences represented normal variants or disease mutations, with Sequencher version 3.3.1 software (GeneCodes). Allelic variations and mutations were queried against public mutation databases (<http://www.eur.nl/FGG/CH1/pompe/index.html> and <http://archive.uwcm.ac.uk/uwcm/mg/search/119965.html>) to determine whether they were novel or had been observed in other patients with Pompe disease.

Extension Protocol

At the conclusion of the 52-week phase, patients were offered enrollment into an extension protocol. During the extension phase, patients received rhGAA at 10 to 20 mg/kg per week or 20 mg/kg every 2 weeks.

RESULTS

Baseline patient characteristics are summarized in Table I. Three patients (D, E, and H) had sibling(s) with classic infantile Pompe disease who had died of cardiorespiratory failure before they reached 1 year of age. All patients had marked cardiomyopathy at presentation. Four patients had the frog-legged posture and “floppy baby” appearance characteristic of patients with an advanced stage of disease progression; 3 of these patients required tube feeding. Although no patients required ventilator assistance at study entry, 4 patients required supplemental oxygen.

Six patients were CRIM-positive by using Western blot; 2 patients were CRIM-negative. The association between CRIM status and the type of GAA gene mutations was examined in 6 patients for whom consent was obtained to perform both analyses (Table II). The 4 patients who were CRIM-positive with available mutation data showed various combinations of missense alleles, deletions, and premature stop codons, indicating that they made detectable but nonfunctional GAA proteins. Of the 2 patients who were CRIM-negative, patient B had a large deletion allele and a frameshift allele; patient H had 2 premature stop codon alleles.

Safety

Treatment with rhGAA was generally well tolerated. Safety data are reported here for the entire study period including the extension (as long as 153 weeks from the first rhGAA infusion). All patients experienced at least 1 AE; most were mild to moderate, were caused by complications of Pompe disease, and were deemed unrelated to rhGAA therapy. Seven of 8 patients experienced at least 1 IAR, including skin rash (urticaria-like, maculopapular, or erythematous), fever, rigors, blood pressure or heart rate changes, or bronchospasm; no IARs were considered severe. IARs were effectively treated by slowing or transiently interrupting the infusion or with acetaminophen, antihistaminics, and/or corticosteroids (administered before infusion or during reactions). No patient had IAR sequelae or discontinued rhGAA treatment because of unmanageable recurrent IARs.

IgG antibodies to rhGAA developed in all 8 patients by week 8 of treatment with rhGAA (Figure 1). After 52 weeks of treatment, anti-rhGAA IgG titers significantly decreased (>4-fold) in patients A and E and remained unchanged in the other patients. Patients F, G, and H each had a single positive test for IgE; all tested negative at subsequent assessments and continued to receive rhGAA without difficulty. No patients had evidence of inhibitory antibodies (capable of inhibiting >10% of rhGAA activity) with an in vitro assay.

Survival

Six of 8 patients completed the full 52-week treatment period. Patients B and G died at ages of 14.7 months and 18.3 months, after 43 and 16 weeks of rhGAA therapy, respectively; neither death was rhGAA-related. Patient B, who started ERT at 4.8 months of age, died after a hospitalization for progressive respiratory distress and pulmonary edema. Patient G, who started ERT at 14.6 months of age with very advanced disease, died of respiratory insufficiency caused by pneumonia and cardiac arrest.

Ventilator-free Survival

After 52 weeks of treatment, 5 of 8 patients were alive without invasive ventilator support, and 1 patient was alive with invasive ventilator support. Patient H became ventilator-dependent at age 11.3 months, after a pneumonia episode occurring 15 weeks after treatment initiation.

Cardiac Response

All patients, regardless of disease stage at study entry, showed sustained improvements in cardiomyopathy as measured by means of LVMI (Table III). At baseline, all 8 patients showed elevated LVMI. The mean improvement rate for the 6 patients with LVMI data at both baseline and week 52 (patients A, C, D, E, F, and H) was 68.7%. Two patients (patients B and G) who died before completing 52 weeks of treatment also showed marked LVMI decreases during treatment (Figure 2).

Motor Response

As shown in Table I, 4 patients (patients B, C, D, and G) entered the trial at a very advanced stage of the disease and were unable to bear weight on their lower limbs. The remaining 4 patients showed severe or generalized hypotonia and head-lag. During the first 52 weeks of rhGAA treatment, 5 patients showed consistent gains in AIMS raw scores and motor-age equivalents (data not shown). The 3 patients with the most marked AIMS gains (patients A, E, and F) ultimately walked independently; the AIMS scores for patients A and E were higher than the 5th percentile for chronological age at week 52. Two patients with more modest AIMS gains (patients C and D) demonstrated improved trunk and upper limb strength and eventually became able to sit independently and roll over, respectively. Patients B and H had only transient gains in AIMS raw scores and motor age equivalents; patient G showed no measurable motor gains at any point.

Mental and Behavioral Development

Although there are no data to suggest that patients with Pompe disease develop cognitive impairment, patients with infantile-onset Pompe disease that is untreated typically do not live long enough or are too ill for cognitive function to be reliably evaluated. Clear and consistent gains in BSID-II mental raw scores and developmental age equivalents were observed in 7 of 7 patients during the first 52 weeks, indicating the continued acquisition of cognitive, language, and personal and social development skills (data not shown). Scores for patient G were excluded because evaluations could not be administered in a standardized manner after the patient was invasively ventilated in an intensive-care unit.

Growth

During the first 52 weeks of treatment, 7 of 8 patients showed continuous increases in body weight and length (Figure 3), resulting in maintenance or improvement in percentile rankings on the basis of Center for Disease Control/National Center for Health Statistics weight and length growth charts. Weight remained unchanged in patient G, whose percentile rank thus dropped by the time of death, 16 weeks after rhGAA treatment began.

Hearing Results

The 3 patients (patients A, E, and F) who eventually walked independently also had normal hearing at baseline and throughout the study, including the extension phase. The remaining patients had abnormal hearing either at baseline (patients D and G) or at various other points, including the extension phase (patients B, C, and H). Frequent middle ear effusions complicated interpretation; however, flat OAE, abnormal wave latencies in BAER, or both suggest that, at least in some cases, there was inner ear, auditory nervous system pathology, or both contributing to these abnormal test results.

Analysis of Skeletal Muscle

GAA activity and glycogen content were measured in the quadriceps muscles of 6 patients at baseline and at treatment weeks 12 and 52. In all 6 patients, muscle GAA activity was lower than detectable limits at baseline, increased substantially after the first 12 weeks of treatment, and remained higher than baseline through 52 weeks of treatment (Table IV). The effect on glycogen in skeletal muscle varied among the patients. Of the patients who walked independently, 1 patient (patient F) showed stabilization of baseline muscle glycogen content and 2 patients (patients A and E) showed marked glycogen reduction. The remaining patients showed either no change (patients B and G; week 12 biopsy) or an increase in glycogen content (patients C, D, and H; week 52 biopsy).

Extension Phase

Six of 8 patients were enrolled into the extension trial, the results of which include evaluations performed after the first 52 weeks of treatment; the total duration of rhGAA treatment (initial plus extension) was as long as 153 weeks. Although in some patients variability in LVMI measurements was observed, all 6 patients showed further decreases in LVMI, which remained significantly lower than at baseline. Similarly, gains in growth parameters after the first 52 weeks of treatment were also observed in all patients who survived. All patients except patient H achieved new motor milestones and maintained previously acquired ones. Patients A and E became able to climb up and down stairs, kick a ball, and ride a tricycle; both are currently >3 years old. Patient F also walked independently. The 4 patients (patients A, C, E, and F) who underwent BSID-II all demonstrated gains in mental raw scores and developmental age equivalents. However, 4 patients died during the extension phase of ERT (patients C, D, F, and H). Patient C died at age 33.8 months after sustaining neurological injury after prolonged resuscitation maneuvers for respiratory insufficiency caused by acute pneumonia. Patient D died of pneumonia and respiratory failure (the family declined invasive ventilation) at the age of 24.8 months. Patient F died unexpectedly at age 32.1 months of a respiratory infection. She had demonstrated marked decreases in LVMI, remained ventilator-free, and was able to walk independently at the time of her death. After becoming ventilator-dependent after an episode of pneumonia, patient H discontinued treatment at the age of 18.5 months in accord with family wishes and died approximately 5 months later. The median age at death (or treatment withdrawal) for all 6 patients who died during the initial 52-week trial and its extension phase was 21.7 months (range, 14.7–33.8 months).

DISCUSSION

In the study by Amalfitano et al,⁸ 2 patients gained and then lost motor skills; the loss occurred at the same time that anti-rhGAA antibodies were detected. These 2 patients were also CRIM-negative. In a third patient who was CRIM-positive, anti-rhGAA antibodies did not develop, and the patient steadily gained motor milestones. In contrast, Van den Hout et al¹¹ did not find an association between CRIM status and anti-rhGAA antibodies or effect on motor development. In this study, 6 of 8 patients were CRIM-positive. Anti-rhGAA antibodies developed in all patients, regardless of CRIM status. As in the Amalfitano study,⁸ at last

evaluation, the 2 patients who were CRIM-negative in this study had only transient motor gains, and anti-rhGAA antibody titers that were among the highest in the group developed. Although no inhibitory antibody activity was detected in these patients by means of an in vitro test, other mechanisms could be involved. The presence of anti-rhGAA antibodies may inhibit rhGAA uptake into cells or alter its biodistribution, as recently demonstrated in immune-deficient GAA knockout mice, an animal model of Pompe disease.²⁵ The effect of anti-rhGAA antibodies on clinical efficacy requires further investigation.

Muscle GAA activity increased markedly after rhGAA administration, but did not always correlate with muscle glycogen reduction. This may result from differences in the receptor-mediated uptake of rhGAA by endothelial cells (for example, in the vasculature) versus skeletal muscle cells within the muscle sample, or inability to clear extralysosomal glycogen in muscle cells severely damaged by advanced disease before ERT inception. The varied effect on glycogen storage may also reflect variable delivery of rhGAA to the skeletal muscle; a study in mice found that a modified form of rhGAA that is internalized to a greater degree than unmodified rh-GAA reduced glycogen storage more than the unmodified form.²⁶ Differences in rhGAA delivery are also one possible explanation for why cardiac tissue responds more consistently to ERT than skeletal muscle: cardiac tissue has more man-6-phosphate receptors than skeletal muscle, thereby providing increased access to rhGAA.^{27,28} Our trial results indicate that glycogen reduction in quadriceps muscle as early as 12 weeks of treatment, or stabilization of a relatively low amount of glycogen content in a longer treatment duration (52 weeks), may predict optimal muscle response to ERT, as seen in the 3 patients who became ambulatory. However, the predictive value of glycogen reduction in quadriceps cannot be generalized to other muscle groups. For example, in this trial, patients starting ERT at an advanced stage of disease acquired significant upper body and trunk motor milestones but did not show glycogen reduction in the quadriceps. Recent studies in GAA knockout mice demonstrated that glycogen clearance after the induction of rhGAA expression became more difficult as the mice aged and the glycogen load became higher.²⁹ Other studies in GAA knockout mice indicate that differences in muscle fiber type and receptor density may affect glycogen reduction in response to administration of rhGAA.^{29,30} Therefore, interpatient variability in motor response and the preferential improvement of upper-limb muscle groups may be influenced by age at first infusion and interactions of different genetic factors.

ERT with rhGAA both extended survival and improved clinical measures in patients with infantile-onset Pompe disease. A subset of patients gained the ability to walk—an achievement unlikely to occur in patients affected with this devastating disease who are not treated. Our results underscore the importance of early treatment to provide the best chance for an optimal motor outcome. This will depend on the prompt recognition of Pompe disease signs and symptoms, ideally before extensive tissue damage develops. In this regard, newborn screening could become important once ERT for Pompe disease becomes available,^{31,32} as could the identification of genetic factors, immunological factors, or both that better predict treatment response.

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Glossary

AE

Adverse event

AIMS

Alberta Infant Motor Scale

BAER

Brainstem auditory evoked response

BSID-II

Bayley Scale of Infant Development, second edition

CHO	Chinese hamster ovary
CNS	Central nervous system
CRIM	Cross-reacting immunological material
ERT	Enzyme replacement therapy
IAR	Infusion-associated reaction
Ig	Immunoglobulin
LVMI	Left ventricular mass index
OAE	Otoacoustic emission
(rh)GAA	(recombinant human) α -glucosidase

Figure 1.

Antibody titers against rhGAA with time. Log dilution titer is shown in the left y-axis; dilution titer is shown in the right y-axis.

Figure 2. LVMI results with time. LVMI was calculated from 2-dimensional echocardiography by a central cardiologist reader.

Figure 3.
Mean weight and length z-scores with time.

Table 1

Patient demographics and baseline characteristics

Patient	Sex	Race/Ethnicity *	Age (months)			Baseline characteristics			
			Symptom onset	Diagnosis	First infusion of rhGAA	LVMI (g/m ²)	Motor	Respiratory †	Tube feeding
A	Male	Asian	1.6	3.8	4.6	352.0	Generalized hypotonia, head lag	No O ₂	No
B	Male	Asian	1.4	4.7	4.8	157.2	Severe hypotonia, head lag, frog-leg position	O ₂	No
C	Male	Caucasian	2.0	6.5	8.0	202.6	Severe hypotonia, head lag, frog-leg position	O ₂	Yes
D	Female	Caucasian	4.8	5.4	8.4	320	Severe hypotonia, head lag, frog-leg position	No O ₂	Yes
E	Male	Asian Indian	0.4	2.5	3.1	188.5	Generalized hypotonia, head lag	No O ₂	No
F	Female	Caucasian	1.4	1.8	2.9	291.9	Generalized hypotonia, head lag	O ₂	No
G	Female	Caucasian	3.3	4.6	14.6	565.2	Severe hypotonia, head lag, frog-leg position	O ₂	Yes
H	Female	Arab	NAV	NAV	2.7	246.67	Generalized hypotonia, head lag	No O ₂	No

Median age in months at first symptoms: 1.6 (range, 0.4–4.8)

Median age in months at diagnosis: 4.6 (range, 1.8–6.5)

Median age in months at first rhGAA treatment: 4.7 (range, 2.7–14.6)

LVM_L, Left ventricular mass index. The mean and upper limit of normal (defined as mean + 2 SD) for LVM_L are 48.8 and 65.0 g/m² in healthy infants (younger than 1 year of age) and 58.6 and 79.2 g/m² in healthy children ages 1 to 4 years.⁶ NAV, Not available.

* As classified by the investigators.

[†]“O₂” refers to supplemental oxygen only. No patients required ventilator support at study entry.

Table II

CRIM status and GAA gene mutation analysis

Patient	CRIM Status	Allele 1 GAA mutation (molecular phenotype)	Allele 2 GAA mutation (molecular phenotype)
A	positive	c.2238 G>A (p.Trp746Stop)	c.1843G>A (p.Gly615Arg)
B	negative	c.148_859-11Del (deletion of exons 2–4, A. Reuser & R. Pomponio, personal communication)& c.1726G>A(p.Gly576Ser)	686 ins CGGC (frameshift)
C	positive	c.1129G>C (p.Gly377Arg) & c.2770T>C (p.Ser924Pro)	c.2560C>T (p.Arg854Stop)
D	positive	Large Deletion Exons 15–20	c.2012T>G (p.Met671Arg)
E	positive	c.1933G>A (p.Asp645Asn)	c.1933G>A (p.Asp645Asn)
F	positive	NAV	NAV
G	positive	NAV	NAV
H	negative	c.2560C>T (p.Arg854Stop)	c.2560C>T (p.Arg854Stop)

Mutations in **bold** have not previously been reported. “c.” indicates the use of NM_000152 cDNA sequence of human GAA as the reference sequence, nucleotide numbering uses the A of ATG translation initiation start site as nucleotide + 1. “p.” represents protein and indicates the predicted aminoacid change resulting from mutation on the basis of a protein reference sequence NP_000143.

Table III

Changes in left ventricular mass index

Patient	LVMI (g/m ²) [*]		Percent change from baseline to week 52 or last assessment
	Baseline	Week 52	
A	352.0	85.5	-75.7
B	157.2	80.7 (at week 34)	-48.7
C	202.6	92.2	-54.5
D	320.0	87.5	-72.7
E	188.5	59.1	-68.7
F	291.9	53.9	-81.5
G	565.1	330.0 (at week 11)	-41.6
H	246.7	124.0	-49.7
Mean ± SD [†]	266.9 ± 64.4g/m ²	83.69 ± 25.33g/m ²	-68.7

Bold indicates within normal limits.

^{*} Normal values for LVMI: for infants <1 year mean = 48.8 g/m², upper limit of normal = 65 g/m²; for 1- to 4-year-olds, mean = 58.6 g/m², upper limit of normal = 79.2 g/m² (15).

[†] For the 6 patients with measurements at week 52.

Table IV

Muscle GAA activity and glycogen content results (quadriceps muscle)

Patient	Time	GAA activity (nmol/hour/g tissue)	Glycogen content (percent area occupied by glycogen; [SD])
A	Baseline	BQL	34.47 (11.06)
	Week 12	49.7	4.25 (2.94)
	Week 52	21.7	2.71 (2.46)
B	Week 4	92.8	40.75 (10.82)
	Week 12	65.9	36.91 (10.36)
C	Week 4	54.8	58.11 (7.59)
	Week 12	28.8	58.17 (6.98)
	Week 52	25.8	70.54 (4.47)
D	Baseline	BQL	42.33 (9.16)
	Week 12	59.8	46.57 (11.43)
	Week 52	40.7	54.43 (11.71)
E	Baseline	BQL	34.98 (8.24)
	Week 12	209	6.88 (5.64)
	Week 52	123.6	0.79 (0.89)
F	Baseline	BQL	25.27 (10.92)
	Week 12	90.1	18.43 (8.27)
	Week 52	21.5	29.95 (9.56)
G	Baseline	BQL	44.61 (10.62)
	Week 12	72.5	40.84 (8.68)
H	Baseline	BQL	ND
	Week 12	83.9	12.03 (4.05)
	Week 52	36.9	42.48 (16.93)

BQL, Below quantitative level (<3.3 nmol/hr/g); ND, not done.