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Biologics Marketing Application
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Recombinant human α -Galactosidase
for
Fabry's disease

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INTRODUCTION

On June 23, 2000, Genzyme submitted an application for marketing approval for their recombinant human α -Galactosidase (r-h α Gal) for the treatment of Fabry's disease. The pivotal trial that Genzyme submitted was based on a primary endpoint of histologically determined reduction in r-h α Gal's substrate in renal interstitial capillary endothelium. This endpoint had been discussed extensively with CBER. Genzyme requested that their product be considered under the accelerated approval mechanism. Under this mechanism, a product may be granted marketing approval by showing a beneficial effect on a surrogate endpoint reasonably likely to predict clinical benefit. However, a trial that attempts to verify the clinical benefit must subsequently be conducted. If such a trial were to show that the product does not confer benefit, the product could be removed from the market. Since submission of the BLA, Genzyme has initiated a randomized, placebo-controlled clinical trial whose design was discussed with CBER before implementation. Genzyme is currently proposing to change the design of this trial from placebo-control to an open-label, single-arm trial with a control consisting of data from a collection of medical histories of Fabry's disease patients.

This briefing document contains a brief description of Fabry's disease and the product. It contains a summary of the major clinical findings presented by Genzyme. It also contains a brief summary of the progress of Genzyme's ongoing clinical trial. A description of the natural history data base, its analysis by Genzyme and a CBER review of these materials are located in a separate document.

FABRY'S DISEASE

Fabry's disease results from an X-linked recessive deficiency in the activity of the enzyme α -galactosidase, an enzyme that catalyzes the release of α -D-Galactose residues from oligosaccharides, galactomannans, and galactolipids. α -Galactosidase A is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) residues on the α -galactosidase A molecule. The M6P moiety binds to a specific M6P receptor in the Golgi and is thus directed to prelysosomal compartments. Enzymes that escape this routing system are secreted by the cell via the constitutive secretory pathway and are often recaptured by cell surface M6P receptors that return the α -galactosidase A to the lysosome by the endocytic pathway.

Many of the clinical signs and symptoms of Fabry's disease are thought to result from accumulation of the enzyme's chief substrate (ceramidetrihexoside, also called GL-3 or Gb3) in vascular endothelium.¹ Glycosphingolipid deposits occur throughout the body; other cell types in which deposition occur include perithelial and smooth muscle cells of the vasculature, histiocytic and reticular cells of connective tissue, epithelial cells of the cornea, glomeruli, and tubules of the kidney, muscle fibers of the heart, and ganglion cells of the autonomic nervous system. Most affected patients are male hemizygotes of western European descent. The incidence of Fabry's disease is approximately 1:40,000 males.¹

Early manifestations of Fabry's disease include pain in the arms and legs (acroparesthesias), vascular skin lesions (angiokeratomata), decreased sweating (hypohidrosis), and opacities in the cornea and lens. Acroparesthesias, which are intense burning pains, usually decrease in frequency with age, but may also increase, and can be debilitating. Paresthesias of the hands and feet may also be present. These forms of pain, although considered the cardinal symptoms of Fabry's disease, may be absent in 10-20% of patients. A partial list of clinical syndromes also includes growth retardation, delay of puberty, lymphedema, diarrhea, anemia, conjunctival and retinal vascular changes, and skeletal deformities. With age, the principal manifestations of concern in Fabry's disease are in the kidney, heart, and brain. Renal disease is manifested by proteinuria, hypertension, and progressive azotemia; the principal cause of death in Fabry's disease is renal failure. Neurological syndromes such as transient ischemic attacks, strokes, seizures, and hemorrhages can occur. Cardiac

involvement may be manifested by left ventricular enlargement, mitral insufficiency, arrhythmias, and myocardial infarction. The median age of death for hemizygous males is 50 years and that of obligate heterozygotic females 70 years.²

Affected males most often have the “classical” form of the disease, as described above, with levels of circulating and cell-associated α -galactosidase activity (hydrolysis of surrogate substrates in a laboratory assay) nondetectable, or nearly nondetectable. Other genetic variants have higher levels of activity, from less than 5% to 35% of normal (normal ranges: 7.8-14.6 nmol/hr/ml (plasma) and 1.8-5.7 nmol/hr/10⁶ cells in leukocytes³). The deficiency of α -gal activity may cause an increase in plasma substrate levels; the normal level is <1.2 ng/ μ l.⁴ Biochemical diagnosis in females is complicated by random X-inactivation. This can raise the overall level of detectable α -gal while levels in critical tissues may be low.

The most commonly described variant in the male is a so-called cardiac variant, whose principal sign is cardiomegaly with or without proteinuria. Patients with this variant may not have the classical early signs of Fabry's disease such as hypohidrosis or pain. Rare female heterozygotes typically have a later disease presentation and a less severe course.

Alpha-galactosidase catabolizes blood group B-specific glycolipids. Persons who are blood group B or AB may be more severely affected due to additional accumulation of these glycolipids.¹

There is no specific treatment for Fabry's disease. Palliative treatments include the anticonvulsants phenytoin, diphenylhydantoin, or carbamazepine for pain, laser for angiokeratoma, anticoagulation for subjects prone to stroke, and dialysis and kidney transplantation for renal failure.

PRODUCT INFORMATION

Genzyme's alpha galactosidase is produced by recombinant DNA technology from an established Chinese Hamster Ovary (CHO) cell line into which the complementary DNA for natural human alpha galactosidase has been inserted. Recombinant human alpha galactosidase is then purified through multiple column chromatography and filtration steps. The enzyme is a homodimer comprised of approximately 50 kDa subunits, and contains three N-linked glycosylation sites. Formulated drug product is supplied as a sterile, lyophilized, white to practically white powder. Each vial of drug product contains 35 mg of Genzyme's alpha galactosidase and is intended for single use. Lyophilized drug product is reconstituted with Water for Injection (WFI) to a concentration of 5 mg/ml and then delivered into sterile saline (0.9% sodium chloride) for intravenous administration.

For preclinical acute toxicity studies and for the phase 1 trial (FB9702-01), r-h α Gal was manufactured at 30 liter and 160 liter bioreactor scales. For the phase III pivotal clinical trial and preclinical repeated dose toxicity studies, Genzyme used a 340 liter bioreactor scale. The proposed commercial manufacturing process will be conducted at the 340 liter scale. For each manufacturing scale, Genzyme demonstrated drug substance comparability through biochemical testing and animal study data.

CLINICAL TRIALS CONDUCTED AND PLANNED AND DATA SUMMARIZED

Table 1 shows completed and ongoing clinical trials by Genzyme. The most important information regarding the safety and efficacy of r-h α Gal is from the results of AGAL-1-002-98, as it is the only controlled data available. AGAL-005-99, the open-label extension to AGAL-1-002-98 in which all subjects receive r-h α Gal, contains important evidence on the durability of the effects seen in the controlled trial.

This document will concentrate on the major findings of FB9702-01, AGAL-1-002-98, and AGAL-005-99. In addition, selected results from AGAL-007-99 and safety findings from additional trials and postmarketing experience will be summarized.

Table 1. Genzyme clinical trials

Trial	n	Design	Status
FB9702-01	15	open-label, dose finding, 5-cohort*	completed
AGAL-006-99	15	Single arm, open-label extension to FB9702-01	ongoing
AGAL-1-002-98	58	randomized, double-blind, placebo-controlled, 5-month, histological endpoint— pivotal	completed
AGAL-005-99	58	Single-arm, open label extension to AGAL-1-002-98	ongoing (over 18 months)
AGAL-007-99	13	Single-arm, open-label, 5-month, histological endpoint	Completed
AGAL-012-01	13	Single arm, open-label extension to AGAL-007-99	Ongoing
AGAL-008-00	73	Randomized, double-blind, placebo-controlled clinical efficacy trial. First subject randomized 2/20/01. Renal, cardiac, and neurologic endpoints.	Ongoing
AGAL-019-01	5	Single-arm, open-label, rechallenge of subjects withdrawn due to IgE	Ongoing

*Only trial using other than 1 mg/kg every other week

Special Note on Review Organization: Numerous tables providing more extensive details of information discussed in this document have been located in the Appendices of the document. All tables from Table 41 onwards are located in the Appendices.

CLINICAL TRIAL DATA

FB9702-01: FIRST, DOSE FINDING TRIAL

The first trial that Genzyme conducted in humans was FB9702-01, called “Pharmacokinetic and pharmacodynamic evaluation of recombinant human α -Galactosidase A (r-h α Gal) replacement in patients with Fabry disease.” It was conducted at Mt. Sinai Medical Center, New York City. It was an open-label, nonrandomized, sequential 5-dose trial in 15 subjects with Fabry's disease, with a duration from 22 to 70 days depending on dose group. Its objectives were to determine the pharmacokinetics and pharmacodynamics of various dose regimens of r-h α Gal and to determine safety.

The results of this trial, given its open-label nature and selection of doses, are less useful than those of AGAL-1-002-98 to follow, but are still informative.

Subjects were to meet the following entry criteria:

Inclusion

- Males with a current diagnosis of Fabry's disease
- ≥ 16 years old
- plasma α -gal activity of ≤ 1.5 nmol/hr/ml
- plasma GL-3 levels ≥ 5.0 ng/ μ l
- clinical presentation consistent with Fabry's Disease

Exclusion

- serum creatinine > 2.5 mg/dl
- have undergone kidney transplantation or currently on dialysis
- clinically significant organic disease, including clinically significant cardiovascular, hepatic, pulmonary, neurologic, or renal disease that in the opinion of the investigator would preclude participation in the trial
- participation in a study employing an investigational drug within 30 days of the start of the trial

Treatment, concomitant medications, and length of evaluation

All subjects received 5 doses. Three groups of three patients each were to receive r-h α Gal at 0.3, 1.0, and 3.0 mg/kg every 14 days; two groups of three patients each were to receive r-h α Gal at 1.0 and 3.0 mg/kg every 48 hours.

Dose levels were based on preclinical information. The every 14-day regimen was chosen to imitate the dosing regimen for Cerezyme, Genzyme's enzyme treatment for Gaucher's disease another inherited enzyme deficiency; the every 48-hour treatment was meant to extend preclinical information based on this dosing frequency and to test the hypothesis that more frequent treatment might result in greater reductions in substrate load.

The product was to be given at 0.83 ml/minute (over 2 hours). There was no limitation on concomitant medications. The final evaluation was to be 2 weeks after the last dose.

Procedures and evaluations

Subjects were screened within 30 days of the first infusion and received baseline enzyme levels and a liver and skin biopsy within 6 days of the first infusion. Periodic assessments of adverse events and laboratories were performed. All subjects were to have liver and skin biopsies at baseline and 2-3 days after the final infusion. Optional second liver and skin biopsies were to be after the 1st infusion for subjects on the 14-day infusion schedule and 14 days after the final infusion for those on the 48-hour schedule.

Biopsy and slide reading considerations

Biopsies were to be processed for light and electron microscopy at Mt. Sinai. Sections for light microscopy were stained with hematoxylin and eosin (liver and kidney), periodic acid-Schiff (liver, skin, and kidney), methylene blue/azure II (skin, heart, and kidney), and/or oil red O (skin). Other sections were made for electron microscopy. Biopsy samples were examined by a Mt. Sinai pathologist specialized to the organ in question, blinded to sample sequence. The degree and extent of glycolipid inclusions were graded on a scale from 0-3 (normal, mild, moderate, and severe) based on an overall judgment of the entire slide. Quantitation of the size and number of glycolipid inclusions was not performed.

Analysis

The final analysis of the trial (safety, pharmacokinetics, GL-3 levels, and clinical outcomes) was to be descriptive.

Comments

FB9702's open-label design rendered it inadequate to give meaningful data on clinical effects of r-h α Gal. The small cohort size rendered its ability to allow conclusions on a dose-relation of findings somewhat tenuous.

Subjects in the every-48-hour spent much less time exposed to r-h α Gal prior to their final evaluations than those in the every-14-day infusion groups. Thus the trial was not designed to give direct information on the durability of the response to r-h α Gal given the more frequent dosing regimen.

RESULTS: CONDUCT OF TRIAL

With a few exceptions, dosing was complete. There were numerous deviations from exact schedules of collection of data and incomplete collection of data, including clinical and pharmacokinetic data. Protocol violations of greatest importance to the outcome of the trial were in the timing of the performance of the liver and skin biopsies, and these were fairly common. The effect that these violations may have had on the results is unknown, as the rate of reaccumulation of substrate, if any, is unknown.

All 15 subjects completed the trial.

RESULTS: DEMOGRAPHICS AND BASELINE CHARACTERISTICS

The trial population was entirely males and mostly "White" (Table 41, appendix). Two subjects were below the inclusionary limit for plasma GL-3. They were in the same treatment group; the mean for all 3 subjects in that group was clearly different from that of any other group. Subjects were mostly in their 4th decade of life. In a few cases serum creatinine was clearly higher than others in the cohort; in most, it was in the normal range.

Comments

The impact of the inclusion of subjects with plasma GL-3 levels below the limit of inclusion is not clear. All subjects qualified on the basis of undetectable α -gal levels.

RESULTS: BIOACTIVITY AND EFFICACY**Histologic GL-3 Assessments**

Liver biopsies were obtained for nearly all subjects; collection of biopsy data in skin, kidney, and heart was more sporadic (Table 42, appendix). This rendered comparisons from the every 14-day to every 48-hour groups tenuous for most organs.

Liver histology

Routine stains used in the liver examination failed to distinguish glycolipid inclusions from background, and thus were uninterpretable. Liver biopsies from several subjects were examined with antibody to GL-3; Genzyme reports that only 2 sets of pre- and post-treatment samples were available, and that they both showed “clearance” of GL-3 after treatment. Photomicrographs from subject 13, presented by Genzyme, appear to show reductions.

Review of scores of transmission electron micrographs of liver sinusoidal endothelial and Kupffer GL-3 were consistent generally with the light microscopic appearance; reductions in hepatocytes in were shown in 7/14 cases where both baseline and end of treatment biopsy data were available. Data on smooth muscle, portal tract vascular endothelium, and ducts were insufficient to make a determination of an effect.

Comment

Liver histology was insufficient to make any conclusions.

Skin histology

Not every specimen received a coded score for light microscopy due to low vessel numbers or uncertain classification on the part of the pathologist. All 7 of the subjects with pre- and post-treatment superficial capillary endothelial scores had reductions to a score of 0 (Appendix Table 43).

Pericyte baseline and end-of-treatment scores were available for 5 subjects. Two of the 5 showed a reduction in GL-3 (with 3 staying the same). Perineurium baseline and end-of-treatment scores were available for 8 subjects. Five of the 8 showed a reduction, with 3 remaining the same.

Electron microscopy evaluation was more consistently done. Electron microscopic evaluations of the endothelium of skin superficial capillaries showed reductions in all 14 subjects who had both pre- and post-treatment scores; reductions were seen in 11/14 in endothelial cells of larger vessels. However, pericyte scores remained the same for 12 of these 14 subjects. No effect was seen in 14/14 pairs of samples of the muscular layer of the arterioles and 13/13 pairs of histiocytes and fibrocytes; while reductions were noted in only 2/13 pairs of perineurium.

Comment

Skin results show that histologically determined reductions in substrate were less robust than the capillary endothelium in some cell types.

Heart histology

As for the skin, both pre- and post-treatment scores were available for the minority of heart samples for light microscopy. Heart substrate levels declined in vascular endothelium as measured by light microscopy in the 7 subjects for whom biopsies at baseline and end-of-treatment were available (Appendix Table 44).

Light microscopy data on cardiac vascular smooth muscle were too limited to draw conclusions; only 2 paired samples were available.

Transmission electron microscopy results for the same subjects' vascular endothelium were consistent with the light microscopy of cardiac vascular endothelium in that for the 7 paired samples examined reductions were seen in all. Genzyme states that histological analysis showed that the bulk of glycolipid in the heart was in the myocytes. Electron microscopy histological evaluation showed a reduction in 1/7 paired samples available. Genzyme performed computer-generated analysis of the volume of cardiac myocytes occupied by lipid. This showed variable results; the 3 subjects in the every 14-day infusion group showed decreases, but 3 of 4 of the subjects in the every 48-hour infusion group showed an increase. Reductions were noted in 4/7 paired evaluations of pericytes, with no change in the others; only 3 paired samples of cardiac smooth muscle were available.

Kidney histology

Because of its importance to the pivotal trial, the results of kidney capillary endothelium are shown in detail (Table 2).

Table 2. Trial FB9702-01: Interstitial capillary scores in kidney (electron microscopic scores in parentheses)

Dose group	Subject	Pre-treatment score	Post-treatment score
3.0/14-day	8	2 (1)	1 (0)
	9	2 (1)	0 (1)
1.0/48-hour	10	2 (3)	1 (2)
	11	1 (2)	2 (0)
3.0/48-hour	13	2 (3)	0 (0)

There was similarly sparse collection of data on other renal cell types (Appendix Table 45).

Pre- and post-treatment scores by electron microscopy (not shown) did not always mirror scores by light microscopy. These scores were not examined in detail.

Comments

There was very little data from this trial in renal interstitial capillary endothelium, but what was available was consistent with results seen in AGAL-002. The data on additional renal cell types showed variable, and sometimes no evident, effect of r-h α Gal treatment.

Overall, available data showed a reduction in lipid in endothelium in the kidney, heart, and skin. Nonvascular cell types had variable, sometimes no evident, reduction in substrate. No dose effect could be discerned from the available data. These data are supportive of an effect in various organs, mostly demonstrated in vascular endothelial cells, but are too sparse to draw firm conclusions as to the amount of reduction one could expect with chronic treatment.

GL-3 levels in tissue biopsies

Total GL-3 levels were determined in biopsies where available (Appendix Table 46). Infusion of r-h α Gal reduced skin and liver total GL-3 levels in almost all subjects. There was no differential effect of dose level or infusion frequency. Baseline GL-3 levels in the liver were quite variable.

Infusion of r-h α Gal reduced GL-3 levels in most renal tissues examined; effects on the heart were quite variable (Table 47).

Comments

Because of the incompleteness of the data, comparisons of the amounts of reduction in histological scores to total GL-3 reduction are difficult to make. The histological data are more useful than total GL-3 in that they detail affected and unaffected tissues in the organs. In general, reduction of total GL-3 occurred for most subjects. The degree of reduction was quite variable, except for the liver.

Plasma GL-3 levels

Levels of plasma GL-3 were reduced on average in all groups by the last infusion (mean reduction range 7% to 70%, without clear dose relationship). Levels of GL-3 in both the 1 and 3 mg/kg dose groups in the every-14-day infusion regimen appeared to drop to end-of-treatment levels by infusion 2.

Urinary GL-3

Genzyme reports that the volumes of urine were inconsistently recorded, so detailed quantification of the GL-3 levels is not possible. The basis for this observation is not detailed.

Other tests reported by Genzyme

The following testing was performed before and after treatment to measure autonomic, renal, and cardiac function, and included quality of life questionnaires. The following summaries are based on summaries and tables in the final report. CBER did not perform an extensive review of individual data.

- *Thermal discrimination testing*
Genzyme tested index finger and great toe thermal discrimination. There was no pattern of improvement or worsening.
- *Sympathetic skin response*
Subjects in the two every 48-hour groups only were tested. There was no consistent pattern of effect.
- *Creatinine clearance*
These data, like the urinary GL-3 data above, depended upon the collection of urine over extended periods of time. According to Genzyme this was not performed consistently. The data show large variability, with some calculated values larger than possible, potentially due to larger collection times than reported. The results are uninterpretable.
- *Renal MRI*
Comparisons between baseline and end of trial were not made by Genzyme, and data were not summarized for review.
- *Echocardiogram*
Mean septal thicknesses and ejection fractions were substantially unchanged between baseline and the 5th infusion.
- *Signal-averaged ECG*
Of the 11 subjects who had baseline and end-of-treatment tests, 10 had normal ECGs that did not change, and 1 had an abnormal ECG that did not change.
- *Cardiac MRI*
Genzyme states that different imaging algorithms were used, even within subjects. This renders comparisons between baseline and end-of-treatment problematic. Comparisons between baseline and end of trial were not made by Genzyme. However, Genzyme noted that there were abnormalities in T2-weighted MRI scans consistent with glycosphingolipid deposition in the myocardium.
- *Ophthalmological exams*
Genzyme does not claim any clinically significant changes due to r-h α Gal infusions.
- *SF-36 Quality of Life Questionnaire (mental and physical separately), Beck Depression Questionnaire, Fabry Quality of Life Questionnaire, Fabry Questionnaire, Impact of Events Scale, McGill Pain Questionnaire (present pain index and number of words chosen), Profile of Mood states (total Mood)*
Virtually no data were reported for the 3 mg/kg every 48-hour group. Genzyme tabulated the data as “better,” “unchanged,” or “worse” without qualifying these labels. Results were not examined in detail. The interpretability of these data is very poor due to the open label nature of the trial.

Pharmacokinetics

Samples for the determination of pharmacokinetic values were taken at infusions 1 and 5. Results are shown in Table 3. The rate of elimination appeared to be biphasic for all dosing regimens and doses. Dose was not proportional to exposure as measured by AUCinfinite (AUCinf) or Cmax as increasing doses yielded higher than proportionate increases in AUCinf and Cmax; furthermore, clearance decreased with increasing dose within any given frequency of dosing. There do not appear to be meaningful differences between pharmacokinetic measures in this study that are dependent upon the numbers of infusions within a dose and regimen:

Table 3. Pharmacokinetic parameters (mean of 1st and last (5th) dose)

Dose group	AUCinf, μ g-min/ml	AUC/Dose	Cmax, μ g/ml	Cl, ml/min/kg	Cl/Dose	T1/2*, min
0.3 mg/kg q14d X 5	76	255	0.6	4.3	14	85
1.0 mg/kg q14d X 5	481	481	4.9	2.7	3	56
3.0 mg/kg q14d X 5	4247	1415	24.7	0.8	0.3	94
1.0 mg/kg q48h X 5	482	482	4.5	2.4	2	58
3.0 mg/kg q48h X 5	2488	829	15.3	1.3	0.4	54

*terminal half-life. The initial elimination T1/2 for the doses 0.3, 1, and 3 given q 14 X 5 were 15 min, 20 min and 45 min.

The development of antibodies to agalsidase occurred in 7 of 15 patients by infusion 5, however, the pharmacokinetic data do not suggest a change in pharmacokinetics parameters.

Summary comments on pharmacodynamic and clinical results

Infusion of the product reduced substrate levels measured biochemically and histologically primarily in the vascular endothelium of various organs—liver, skin, kidney, and heart. There was no clear evidence of differential effect of the different infusion regimens. There were no notable clinical beneficial effects.

RESULTS: SAFETY**Exposure**

Nearly all subjects completed all of their infusions.

Deaths

There were no deaths in this trial.

Adverse events: serious adverse events

There were 2 subjects with serious adverse events:

Patient 5 (1.0 mg/kg every-14-days) experienced a serious infusion reaction at infusion 4 requiring cessation of infusion and medical treatment (see the section on infusion reactions).

Patient 14 (3.0 mg/kg every-48-hours) had pulmonary emboli. There are no other data to suggest thrombotic events, and the clinical data for this case do not suggest a concern for thromboembolic events.

Infusion reactions

Infusion reactions were reported in 4 subjects, 3 of whom were in the every-14-day 3 mg/kg dose group. Table 48 shows outlines of the events, including laboratory testing. Serum anti-r-h α Gal IgG was detected in all subjects with a reaction, but serum IgE was not detected in 3/3 tested.

Comments

All infusion reactions occurred after several uncomplicated infusions had been given. For two of the subjects, a subsequent infusion had to be stopped. Genzyme slowed the rate of infusion in the subsequent trial, AGAL-1-002-98, and pretreated all subjects. For a further discussion of infusion reactions and hypertension, see the safety section of the review of AGAL-1-002-98.

Although IgG was detected in all those with infusion reactions, it was also present in some subjects without significant reactions (see Table 48), so that it is not a useful prognostic feature.

Other adverse events

The most consistent adverse event was hypertension, occurring in 14/15 subjects, all starting on an infusion date.

Other adverse events were sporadic, showing no particular pattern among the dose groups.

Comments on adverse events

Hypertension was the most important adverse event other than infusion reactions in this trial. Although it was common, it was not severe. This adverse experience was not seen as an isolated event in AGAL-1-002-98 or its extension (see discussion of hypertension in the AGAL-1-002-98 review).

Concomitant medication use

The pattern of this and other medication use overall was not remarkably different from that expected in the Fabry's population.

Summary (safety)

The most important safety concern in this trial was the occurrence of severe hypersensitivity-type infusion reactions and hypertension. Although antibody to the product was found in all the subjects who had infusion reactions, this was not a prognostic factor since several subjects with antibody did not have reactions. Hypertension seemed related temporally to the administration of product, but was not severe.

Antibody production

The majority of subjects ended the trial with antibody to the product, consistent with results in Study AGAL-002. Ten of 15 subjects ended the trial with serum IgG to r-h α Gal by ELISA; 9/15 by Western blot (1 had a pre-existing antibody reactivity by ELISA, and 3 had pre-existing antibody by Western blot).

The numbers of subjects is too small to draw any conclusions about the relationship of dose to the development of antibodies.

CONCLUSIONS REGARDING FB9702-01

This trial was not designed to show efficacy. It showed that Genzyme's r-h α Gal can reduce GL-3 inclusions in the capillary endothelium of various organs, but did not show reductions in some other cell types in various organs. The largest safety concern was the presence of infusion reactions in some of the subjects, which occurred despite premedication.

Genzyme concluded that the every 14-day regimen resulted in the most consistent reductions in substrate levels, and chose to conduct a controlled trial to study the 1 mg/kg dose level due to a lower incidence of infusion reactions than shown at the 3 mg/kg level.

AGAL-1-002-98: CONTROLLED TRIAL

Genzyme's Study AGAL-1-002-98, was entitled "A multicenter, placebo-controlled, double-blind, randomized study of the safety and efficacy of recombinant human α -Galactosidase (r-h α GAL) replacement in patients with Fabry Disease." It was a multinational, double-blind, randomized, placebo-controlled, multiple-dose trial in individuals with Fabry's disease. Its primary objectives were to determine activity in terms of reduction of enzyme substrate from kidney, heart, and skin tissue, and to assess safety. As mentioned in the Introduction, the endpoint had been extensively discussed with CBER before the trial was implemented.

This trial, because of its design, is the primary evidence of bioactivity, efficacy, and safety.

Treatment

Subjects were to be randomized either to receive the product, 0.9-1.1 mg/kg, or placebo (mannitol with a phosphate buffer) every 2 weeks by intravenous infusion for 24 weeks (a protocol amendment lowered this to 20 weeks). Due to concerns about infusion reactions seen in FB9702-01 trial agent was to be infused more slowly, at no more than 0.25 mg/min (over 4-6 hours). In addition, all subjects were to be pretreated with acetaminophen 975-1000 mg and hydroxyzine 25-50 mg orally. The product and placebo were to be administered as slow IV infusions. The composition of the placebo was the same as that of the product, without the enzyme (3% mannitol in 50 mmol sodium phosphate buffer).

Randomization and blinding

Subjects were to be randomized in balanced blocks stratified by site. Placebo and active treatments were blinded.

Subject qualifications

Subjects were to meet the following entry criteria:

Inclusion

- ≥ 16 years old
- current diagnosis of Fabry's disease
- clinical presentation consistent with Fabry's disease
- no prior treatment with r-h α Gal
- plasma α -Gal activity of < 1.5 nmol/hr/ml or leukocyte α -gal activity of < 4 nmol/hr/mg
- negative pregnancy test (urine β -hCG) prior to dosing at each study visit (female patients of childbearing potential)

Exclusion

- Current evidence of kidney failure or renal insufficiency, as defined by a serum creatinine > 2.2 mg/dl (194.7 μ mol/l)
- Receipt of kidney transplantation or current dialysis
- Clinically significant organic disease (with the exception of symptoms relating to Fabry disease), including clinically significant cardiovascular, liver, pulmonary, neurologic, or renal disease, or other medical condition, serious intercurrent illness, or extenuating circumstance that, in the opinion of the investigator, would preclude participation in the trial
- Participation in a study employing an investigational drug within 30 days of the start of the trial
- Pregnancy or lactation

Procedures and evaluations

The following is a synopsis of the important procedures specified in the protocol. The first infusion was to be at Visit 1 on Day 0.

- Baseline assessments were to be completed after enrollment and within 28 days prior to the first infusion:
 - Medical and surgical history, physical examination
 - Clinical chemistry, hematology, serum cystatin C, and urinalysis
 - 12-lead ECG and echocardiogram
 - Leukocyte α -gal
 - r-haGal antibody
 - GL-3 level and plasma α -Gal activity
 - Biopsy of heart, kidney, and skin
 - Short Form McGill Pain Questionnaire, SF-36 Health Survey, Fabry Symptom Assessment, Neuropathy Impairment Score (U.S. patients only), Neuropathy Symptoms and Change Score (U.S. patients only), Total Symptom Score
 - Neurophysiological function testing (U.S. patients only)
 - Ophthalmic examination
 - 24-hour urine GL-3
 - Glomerular filtration rate by para amino hippuric acid (PAH) and inulin clearance
 - Dispensing of daily patient diary
 - Adverse event and concomitant medication monitoring
- Randomization was to occur after the above procedures had been completed.
- Visits were conducted every 2 weeks, with antibody assessments, infusions, reviews of diaries, adverse event and concomitant medication assessments.
- Subjects received periodic routine laboratory assessments
- At the final visit (5-months, visit 11), the following were performed:
 - Trial agent infusion
 - r-haGal antibody
 - Clinical chemistry, hematology, serum cystatin C, and urinalysis
 - Pharmacokinetics (European patients only)
 - Vital signs
 - Plasma for GL-3 (prior to study drug infusion)
 - Collection and review of patient diary
 - Short Form McGill Pain Questionnaire, SF-36 Health Survey, Total Symptom Score, Fabry Symptom Assessment
- Following the final visit, there was a followup visit, with a 28-day window, where the following were performed:
 - Physical examination
 - Biopsy of heart, kidney, and skin
 - Adverse event and concomitant medication monitoring
 - 12-lead ECG and echocardiogram
 - Neurophysiological function testing, Neuropathy Impairment Score, Neuropathy Symptoms and Change Score (U.S. patients only)
 - Ophthalmologic examination
 - 24-hour urine GL-3 (This could be obtained up to 14 days prior to Visit 11, but after the Visit 10 infusion.)
 - Glomerular filtration rate by PAH and inulin clearance

- Standard urea and creatinine clearance tests, including 24 hour urine and serum chemistries, only on patients who had not undergone inulin clearance testing. The protocol allowed samples to be collected for testing after the 28 day window.

Study sites were to attempt to reach all trial participants by telephone approximately 2 weeks after the final follow-up procedures for a safety call.

In summary, biopsies and most measures of efficacy were to be performed before the first infusion and after the last infusion. Subjects completed a daily symptom diary and were monitored for adverse events and concomitant medications during the trial. Clinical laboratories were measured at baseline, day 84, and the end of the trial.

Biopsy procedure and histology slide blinding and preparation

The trial operations manual specified that the kidney biopsy site be located by means of ultrasound to the lower pole of either kidney and that 2 cores be obtained if possible, avoiding diseased areas. For the heart, 8 small pieces of tissue from the right ventricular endomyocardium were recommended; for the skin, two 3 mm punch biopsies or the equivalent amount of tissue, avoiding angiokeratomata, were recommended from the lower flank/upper buttock. Preserved or frozen tissue samples were to be shipped to Genzyme, identified by subject and collection date. Genzyme personnel masked the biopsies with respect to subject identity and trial visit using a prespecified list and shipped them to Mt. Sinai Medical Center's histology laboratory.

Kidney biopsy samples were sectioned and stained at Mt. Sinai with methylene blue/azure II for light microscopy and reviewed by Mt. Sinai histology technicians for staining and tissue quality (for example, darkness of stain and presence of wrinkles or tears). Rejected samples required new thin sections to be prepared. The final quality review was performed by the Mt. Sinai Director of Pathology. A rejection by the Director required a further thin section to be made, starting the quality review process again. Accepted slides were returned to Genzyme. Dr. Richard Diters, an employee of Genzyme, rendered a final judgment on the histological readability of slides under low power microscopy. Accepted slides were sent to reviewing pathologists. Genzyme states that Dr. Diters was not involved in the design or management of the trial, and had no knowledge of the trial blind or timing of sample collection.

Comments on blinding

Genzyme's detailed procedures kept slide reviewers from knowledge of subject treatment assignments and trial visit. The only members of Genzyme whom the procedure unblinded were members of Quality Assurance and the personnel who created the masking list. The procedure appears adequate to assure the integrity of the review process.

Analysis

Analytical populations

Genzyme prospectively defined 3 subject populations for analysis (the intent-to-treat, as-treated, and per protocol, populations).

Comment

Although 3 populations were defined by Genzyme, this document will concentrate on one population (the "effective" intent-to-treat, in this case similar to the as-treated group).

Primary endpoint

The primary endpoint was based upon quantitation of GL-3 inclusions in the capillary endothelium (vasculature) of the kidney as determined by light microscopy.

The primary endpoint was a comparison of the number of subjects in each group who achieve a score of Zero on the renal capillary endothelium histology.

Scoring method for endothelium

Kidney interstitial capillary endothelium was initially evaluated by 3 pathologists using a qualitative method that had not received concurrence from CBER. Slides received scores of 0, 1, 2, or 3 (“nearly none” or “trace,” “mild,” “moderate,” or “severe”) based on an overall impression of the amount of GL-3 present, and a majority score was recorded (with a procedure for resolving discrepant scoring among pathologists).

In discussions with Genzyme, CBER raised concern regarding the reliability and interpretability of the global, qualitative scoring system. Subsequent to those discussions, Genzyme had a subset of the slides re-read by the pathologists using a well-defined quantitative scoring procedure. The following are important aspects of the scoring:

1. Slides from the initial, nonquantitative read that were scored as 0 or 1 were the only ones subject to the quantitative rereading procedure. Since only slides with scores of zero contributed to the endpoint “success” category, it was assumed that any slide that had received an overall impression of mild (i.e., clearly present inclusions) or worse would not achieve a score of zero on the re-read.
2. Capillary score: All capillaries on each slide were individually examined and received grades of 0, trace, 1, 2, or 3, based on imaging within the endothelium at 1000x with oil immersion:
 - “0” signified no visible inclusions
 - “trace” signified a single inclusion, or 2 inclusions for capillaries cut in such a way that the length of the lumen was greater than twice the width
 - “1” signified multiple discrete lipid granules
 - “2” signified single or multiple aggregates of lipid granules
 - “3” signified aggregates of lipid granules either large enough or numerous enough to cause clear distortion of the luminal surface

The entire section was scanned field by field under the microscope.

3. Slide score: A new slide score was generated for each slide, based on this complete list of capillary scores on the slide. Slides were given a score of 0 or 1, where 0 was interpreted as “nearly clear” of capillary inclusions and 1 was “not clear.”
 - a. 5% of the total capillaries per slide with the highest scores were discounted from the analysis. Thus a slide with an eventual score of “nearly clear” could have theoretically had 4.9% of its capillaries scored as high as 3.
 - b. The remaining capillaries were summarized by the number with each capillary score
 - c. A slide score of 0 would be obtained if
 - More than 50% of the remaining capillaries were free of GL-3 inclusions (capillary score 0)
 and
 - The remaining capillaries contained capillaries with no higher grade of inclusions than “trace.”
 - Otherwise the slide reread score was “not zero.”
 - d. All three pathologists would generate a slide score in this manner. The slide would receive the score that the majority of pathologists gave it.

Comment On Endpoint Definition and Selection:

In essence, this system classified as “success” (score of 0) slides where, after disregarding the worst 5% of capillaries on a slide, more than 50% of the capillaries had no light microscopy visible inclusions, and all the rest had no more than trace inclusions.

The underlying basis for this endpoint is important to understand. This study was prospectively intended primarily as a study to provide evidence regarding a surrogate endpoint in order to seek marketing approval under the Accelerated Approval mechanism. After being unable to provide good rationale why plasma levels urinary levels of substrate alone could be justifiably regarded as “reasonably likely” to predict clinical benefit, Genzyme selected the renal capillary endothelium as the histological site to evaluate the effect of enzyme administration.

Genzyme sought extensive discussions with CBER regarding an endpoint based on the capillary histology that might support use of the Accelerated Approval mechanism. CBER raised concerns that any specific quantitative reduction in endothelium inclusion volume (e.g., 50%, 75%, even 90%) might not result in benefit if there was a threshold phenomenon, where the remaining volume of inclusions were still sufficient to lead to vascular injury. Genzyme was unable to locate any data that could establish a level of inclusions that did not lead to vascular injury. Consequently, Genzyme proposed that restoration of capillaries to near-normal appearance overcame the concerns regarding a threshold process, and could be regarded as “reasonably likely” to predict benefit. CBER agreed that this criterion appeared to provide a much stronger case for prediction of benefit.

In addition, as a result of CBER concerns over the cell-type generalizability of the histological effect, additional cell types were examined by the same pathologists at a later time. These results are discussed in this briefing document in the review of the extension trial, AGAL-005-99.

Analytical methods and missing values

Genzyme followed their prospective plan for analysis. See the results section.

Secondary endpoints

Secondary endpoints were as follows:

- 1) Pain as assessed on the McGill short form pain questionnaire in 5 domains (sensory, affective, total, visual analog scale, and present pain intensity).
- 2) Composite of capillary endothelium scores of the heart, kidney, and skin as determined light microscopy comparing baseline to 20 weeks
- 3) Total GL-3 measured by ELISA in urine and kidney tissue

Tertiary and “other” endpoints

These are mentioned in the results section of this briefing document.

Comments on the protocol

Genzyme's previous results suggested that a brief trial such as this one (6months) could demonstrate differences from placebo treatment on the chosen surrogate primary endpoint. Given the expected nature of the treatment effect, the trial's brevity and small size rendered it more problematic that subjective measures such as pain and symptom scores would show relevant, meaningful differences, despite the use of placebo controls. The final endpoint was one agreed to in discussions with CBER, based on a reasonable likelihood that it would predict a clinical benefit and that a confirmatory trial would be conducted.

The chief evaluations, the biopsies, were performed only at the beginning and the end of the trial, making a detailed assessment of the kinetics of a possible histological effect impossible.

RESULTS: CONDUCT OF TRIAL

Protocol changes

The protocol underwent significant revisions after the trial was started (March 14, 1999). Amendments increased enrollment from about 50 to about 60; shortened the trial by one month; changed the endpoint from a composite of histology of skin, heart, and kidney combined to the quantitation of GL-3 in kidney alone; and finalized the agreed-to rereading of slides by the quantitative method as the primary analysis.

Comments

The changes to the protocol were not prompted by review of the data, and thus do not raise suspicions as to the integrity of the results of the trial. While the changes to the analytic plan and endpoint occurred well after the trial started, and were not finalized until after data collection was completed, all revisions were made while Genzyme was fully blinded to any study outcome data.

It is notable that the study was shortened. Subjects received one month less of infusions than initially planned. No clear scientific rationale was provided by Genzyme for this change.

Enrollment by site

Fifty-eight subjects were enrolled. The Mount Sinai site had the most subjects by a large margin; 20 subjects were enrolled there, with a range of 2-9 at the other 7 sites (Table 49).

Discontinuations

No subject discontinued participation in the trial.

Protocol violations

Treatment assignments

The most important protocol violation was the reversal of treatment assignments for 4 subjects at site 7, and partial reversal for 2 subjects at site 5 (Table 4).

Table 4. Trial AGAL-1-002-98: Errors in treatment assignment

Site	Subject ID	Intended treatment assignment	Treatment Received
5	0503	product	3 infusions product, then 8 placebo
	0504	placebo	3 infusions placebo, then 8 product
7	0701	product	placebo
	0705	placebo	product
	0706	placebo	product
	0708	product	placebo

Additional information was supplied by Genzyme regarding these errors in randomization and/or administration. A contractor for Genzyme for preparing and dispensing of study treatment kits failed to inscribe some kits with the Genzyme-supplied subject identification numbers on the first kit for study subjects for use at sites 5, 6 and 7 (study sites would receive additional kits for the study subjects for the sequential infusions). Genzyme was notified of this error and able to correct it prior to use of kits at study sites 5 and 6.

At site 5, correct kits were used for subjects 503 and 504. However, it was noted that Subject 504 was actually treated prior to Subject 503, and concern that the treatment kits had been reversed for the two subjects on the part of the site was communicated to Genzyme. This led to a switch in treatment material supplied to the site in the understanding that this would maintain constant treatment receipt in these two patients. After the trial was completed it was discerned that miscommunication and misunderstandings had occurred. The initial treatment received had been the correct intended, and the adjustment had caused an switch in actual treatment received.

At site 7 inadequately marked initial kits were used for subjects. Kits were used without knowledge of kit contents, but were trackable based on other code numbers on the kit. Consequently, the treatment assignments at site 7 can be regarded as randomized, but not according to the centrally planned randomization scheme. Of 9 patients enrolled at this site and subject to the problem, 5 received treatment kits other than what was centrally planned, but containing the same material as was centrally planned. There were 4 subjects who received an initial treatment kit different from the centrally planned and which contained the opposite treatment from the centrally planned (2 each switched in each direction, as noted in Table 3). At this site, subsequent treatment kits were adjusted to maintain the same material as in the original treatment kit. Documentation supplied by Genzyme established that no unblinding information on kits had been opened.

The most important other protocol violation concerned filtration and centrifugation of urines at both sites in France, rendering urinary results at those sites unevaluable.

Comments

Treatment misassignments apparently occurred due to an error that was related to contractor errors and localized to certain sites. These problems were not reflective of overall trial conduct, and they were made without unblinding treatments. There is no evidence of a systematic bias in treatment assignments. Since the subjects at site 7 can be regarded as randomized without bias, analysis of the outcomes of the subjects at site 7 can be included in an as-treated basis but considered an "intent-to-treat" analysis. Subjects 503 and 504 can be included as well, since they received a substantial majority of their infusions (8/11) with a consistent material, and toward the end of the trial. Thus, subjects 503 and 504 have been included in analyses according to the majority of their treatments. This analysis can be considered the best feasible, least biased analysis of these data.

Adherence to dosing

Adherence to trial drug infusion and dose amount was excellent.

RESULTS: DEMOGRAPHICS AND BASELINE CHARACTERISTICS

There was no evidence of screening bias.

There were no major demographic differences between treatment groups (Table 5).

Table 5. Trial AGAL-1-002-98: Demographics

Parameter	Statistic	Placebo n=29	r-h α Gal n=29
Age (yr)	Mean	28.4	32
	Min., Max.	17,61	16,48
Weight (kg)	Mean	69.6	67.3
	Min., Max.	46,96	50,86
Height (cm)	Mean	175.6	175.7
	Min., Max.	156,203	158,196
Gender: Male Female	n (%)	29 (100)	27 (93)
	n (%)	0 (0)	2 (7)
Race: White Non-White	n (%)	26(90)	27(93)
	n (%)	3(10)	2 (7)

The only two females in the trial were in the active-treatment group and the great majority of subjects were categorized as “White.” The mean age of the placebo group was slightly lower than that of the active group, but there was no major difference in the shape of the age distributions in the two groups (not shown).

Baseline characteristics of the subjects were balanced (Table 6).

Table 6. Trial AGAL-1-002-98: Baseline characteristics

Parameter	Statistic	Placebo	r-h α GAL
Plasma α -Gal activity (nmol/hr/ml)	<i>n</i>	29	29
	Mean	0.49	0.65
	Min., max.	0, 1.5	0, 1.5
Plasma GL-3 (ng/ml)	<i>n</i>	29	29
	Mean	14.4	14.7
	Min., Max.	0, 36	0, 36
Serum creatinine	<i>n</i>	29	29
	Mean	0.79	0.83
Years since onset of symptoms	<i>n</i>	29	28
	Mean	21.2	23.4
	Min., Max.	9,55	8,44
Years since initial diagnosis	<i>n</i>	28	28
	Mean	9.9	10.6
	Min., Max.	0,46	0,28
Blood type	<i>n</i>	29	29
	A or O	27	27
	B or AB	2	2

Endogenous baseline plasma α -Gal activity was below the level of detection in all subjects (leukocyte α -Gal activity was collected after baseline in some subjects and is not useful for comparison). The presence or absence of the B-specific blood group antigen was equal between the treatment groups. Thirty-seven genotypes were identified. Among these, only 5 were shared by a subject in both treatment groups, none by more than one subject in either group. In addition, no genotype was found more than 3 times in any treatment group. Baseline characteristics were balanced, and the genotype diversity precludes any conclusions about differences between groups.

RESULTS: BIOACTIVITY AND EFFICACY

Analytical population

As discussed in the description of treatment assignment errors, the “as treated” group (including partially treated subjects 503 and 504) can be appropriately considered as a valid least-biased analysis grouping. This review will focus on this “as-treated/intent-to-treat” population. Genzyme also submitted efficacy analyses for the trial population excluding subjects 503 and 504; review of primary, secondary, and tertiary endpoints and plasma GL-3 data (an “other” endpoint) did not lead to different conclusions from those presented here.

Primary endpoint

At baseline there was a weak trend toward greater severity in the placebo group (Table 7).

Table 7. Trial AGAL-1-002-98: Distribution of baseline kidney slide scores*

Slide score	Placebo	r-h α Gal
0	0	1
1	4	7
2	15	14
3	10	7
<i>Total</i>	29	29

*as-treated population, considered equivalent to intent-to-treat

Table 8 shows the data and analysis of the primary endpoint: morphological appearance of inclusions in the kidney vasculature. The p-value is based upon the prospectively-defined χ^2 test, and includes an imputed nonzero score for subject 307 in the r-h α Gal-treated group.

Table 8. Trial AGAL-1-002-98: Primary endpoint results: Renal capillary endothelium inclusions*

End of trial Score	Placebo <i>n</i> =29	r-h α Gal <i>n</i> =29
Zero	0 (0%)	20 (69%)
Non-zero	29 (100%)	9 (31%)
Odds ratio (C.I.)	0.008 (0.00, 0.14)	
p-value	<0.001	

*as-treated population, considered equivalent to intent-to-treat

Table 9 shows details of the end-of-trial scores used to assign “zero” or “nonzero” status. The p-value is based on a 2x4 χ^2 test.

Table 9. Trial AGAL-1-002-98: Distribution of end-of-trial slide scores*

End of trial score	Placebo <i>n</i> =29	r-h α Gal <i>n</i> =29
0	0 (0%)	20 (69%)
1	7 (24%)	8 (28%)
2	11 (38%)	0 (0%)
3	11 (38%)	1 ** (3%)
p-value	<0.001	

*as-treated population, considered equivalent to intent-to-treat

**Subject 307, attributed a worst-case score in the absence of an end-of-treatment biopsy

These data show that in the active-treated group, end-of-trial nonzero scores were only in the mild category (excepting the one purely imputed score), but that in the placebo group scores were distributed among mild, moderate, and severe. The results show that the effect of r-h α Gal was to diminish renal capillary endothelial GL-3 regardless of baseline severity.

The results of Genzyme's ANOVA analysis are consistent with these results.

Review of transmission electron microscopy

During the second, quantitative reading of the kidney slides pathologists assessed transmission electron photomicrographs. In no case was a discrepancy noted, showing that electron microscopy evaluation was consistent with the light microscopic appearance.

Genzyme's exploration of primary endpoint

The following section describes important additional analyses conducted by Genzyme to examine the robustness of their primary results.

Baseline scores for subjects with treatment errors

Baseline histological severity of the subjects with treatment assignment reversals were distributed similarly (1 each at 1, 2, and 3). This supports the view that there was no bias in the treatment (mis-)assignment related to baseline severity.

Distribution of baseline scores among those who ended trial with a “0” score

The activity of the product was not limited to those with lower baseline kidney slide scores. The majority of subjects who achieved a score of “0” at the end of treatment had baseline scores of “2” or “3.” (Table 50).

Consistency of the pathologists in scoring

Genzyme tabulated the proportion of “0” scores by pathologist for both the intent-to-treat and the as-treated population. All pathologists gave the active-treated groups many more “0” scores.

The proportion of capillaries with a capillary score of “0” was almost uniformly more than 90% for each pathologist (Genzyme analysis done on the absolute-intent-to-treat group).

Dr. Rennke scored the most capillaries in a given biopsy in almost all cases, sometimes by 100 or more. Dr. Rennke’s greater number of capillaries scored did not render his overall result discrepant from the others; Dr. Colvin, not Dr. Rennke, was the pathologist whose number of zero scores differed most from the rest (Dr. Rennke scored 21/29 with a score of “0,” Dr. Colvin 12/29, and Dr. Dikman 27/29).

Sensitivity of Score to disregarded capillaries

Genzyme’s scoring system allowed for an arbitrary 5% of the worst capillaries to be excluded from consideration. Genzyme proposed this procedure in the prospective design due to concerns that a small number of aberrant capillaries might prohibit a dramatic effect on the overall biopsy from being demonstrated. The 5% value was arbitrarily chosen.

Genzyme examined the slide score results if alternative disregard criteria were used. When a more stringent criterion of 1% disregard was used, there were only 10 galactosidase subjects achieving a score of 0, vs. none in the placebo group. When a more liberal criterion of 10% was used, overwhelming predominance of the “0” score in the galactosidase group remained (24 vs. 3 using the absolute-intent to treat grouping).

Comment: While the 5% criterion is arbitrary and may be too liberal an amount of capillaries to disregard, the finding of treatment associated difference in histological outcome is a consistent finding. The fraction of subjects who achieve “success” is altered by the 1%-5%-10% criterion, and thus possibly the fraction of patients who may ultimately benefit. The observation of a treatment related effect is a highly robust finding.

Effects of age on primary endpoint

Genzyme stratified the endpoint analysis at the median age of the overall population, 30 years. The analysis of age is important since Fabry’s disease is a congenital disorder and duration of disease is the same as age. Table 10 shows that the active treatment was effective regardless of the age group, although there does seem to be a slightly diminished effect in the older group (see CBER’s sensitivity analyses). Older subjects did not have a greater baseline severity (not shown).

Table 10. Trial AGAL-1-002-98: Effect of age on the primary endpoint*

Age stratum (years)		Placebo	r-h α Gal
<30	Zero	0	9 (82%)
	Non-zero	18 (100%)	2 (18%)
	p-value	<0.001	
30+	Zero	0	11 (61%)
	Non-zero	11 (100%)	7 (39%)
	p-value	0.001	

*as-treated population, considered equivalent to intent-to-treat

Effects of ethnicity and gender on primary endpoint

The number of “non-White” subjects was very small (5), and there were only 2 women in the trial. No conclusions can be made regarding ethnicity or gender from this small amount of data.

Effect of trial site on endpoint

Results were generally consistent from site to site (Table 51). Although center 1 had the most subjects, it did not alone produce the favorable results. Other centers had results fully consistent with those seen at site 1. Centers 3 and 5, with a total of only 1 slide scored as a “0” in 6 r-h α Gal-treated subjects, did not contribute to the overall effect.

CBER's exploration of the primary endpoint

Table 11 shows the numbers of subjects with specific kidney slide score changes from baseline, tabulated by treatment group and baseline score. This analysis shows numbers of subjects with scores of “0” at the end of the trial and change in score as a function of baseline score. Change score is potentially a more sensitive indicator of the effect of treatment than achievement of a criterion value. In addition, this analysis, when carried out on the placebo group (which is not expected to change appreciably on a laboratory criterion in a brief trial such as this one), allows examination of the variability of the scoring procedure.

Table 11. Trial AGAL-1-002-98: Numbers of subjects with specified changes from baseline score, by baseline score, on “as-treated” population (bold signifies numbers of subjects who achieved a score of “0” at the end of the trial)

Group	Baseline score	Change from baseline						total
		better				worse*		
		-3	-2	-1	0	1	2	
Placebo	0				0	0	0	0
	1			0	1	1	2	4
	2		0	3	5	7		15
	3	0	3	5	2			10
	total	0	3	8	8	8	2	29
r-hαGal	0				1	0	0	1
	1			3	3	0	1	7**
	2		11	3	0	0		14
	3	5	2	0	0			7
	total	5	13	6	4	0	1	29

*There were no worsenings by greater than 2

**This includes subject 307, who failed to have a biopsy at the end of trial, and who was attributed a worst-case score of 3 at the end of the trial.

Among the placebo subjects the overall distribution of change scores was centered around 0, with approximately equal numbers of slides getting worse or better by 1 or 2 points. The fact that a moderate number of placebo subjects changed by 1 point in either direction (and in fact most subjects did not remain at the baseline score) shows that a change in score by 1 point does not

reliably indicate a true change. However, more than one half of the improvement in scores in the active group were of magnitude 2 or 3, which is reliably measured change.

Localization of biopsy tissue

A small number of biopsies were of medullary tissue, not cortical tissue. These results of these biopsies were consistent with those of cortical tissue.

Subgroup analysis of the primary endpoint (age)

The increased proportion of nonzero scores in the older age group (see Genzyme's analysis above) is not due to a greater number of subjects with most severe disease, as the distribution of baseline scores in the two age groups is similar (not shown).

When the r-h α Gal-treated subjects are analyzed by study quartiles of age, there is no age-related pattern of change score from baseline (Table 12). Note that the age distribution was not identical in the two groups. The r-h α Gal-treated subjects were slightly older, so that there are more subjects in the 30-40 yo subset than other subsets within this group. The apparent greater activity of the enzyme in the younger subjects suggested by the Genzyme analysis above is not borne out by a more subdivided analysis.

Table 12. AGAL-1-002-98: Change Score by age in r-h α Gal-treated subjects

Age	Change Score (-) signifies improvement)					p-value*
	-3	-2	-1	0	+2	
≤ 21.5	0	3	1	1	0	0.94
(21.5, 29.5]	3	1	1	0	1	
(29.5, 40]	1	5	2	2	0	
> 40	1	4	2	1	0	

* Jonckheere-Terpstra test

Other CBER subgroup analyses of the primary endpoint

The CBER statistical reviewer examined the distribution of change scores as a function of quartiles of baseline plasma GL-3 and kidney GL-3 and when dichotomized at the median of urinary GL-3. There was no notable pattern of change scores using any of these parameters (analyses not shown in this document).

Independent pathologist's evaluation of primary endpoint

CBER requested an assessment of the kidney biopsy slides from J. Charles Jennette, M.D. Dr. Jennette is a renal pathologist and Professor and Chair of Pathology and Laboratory Medicine at the University of North Carolina School of Medicine. CBER asked Dr. Jennette to examine a subset of all the kidney slides, containing the full range of scores in each treatment group. He concluded that the scoring of lipid inclusions was "relatively reproducible" and that "the specimens can be accurately grouped on the basis of the relative extent of lipid accumulation in peritubular capillaries." Dr. Jennette's slide scores shows a good correspondence to the scores given by the renal pathologists trained and employed by Genzyme (Table 52).

Comments on primary endpoint

The activity of r-h α Gal on renal interstitial capillaries was robustly shown in this trial. Subgroups of gender and ethnicity were too small to render useful information. Based on a small number of subjects, there may have been a small diminution of effect with increasing age, but this suggestion is not fully supported by further sensitivity analyses. The overall results were not driven by site, but occurred nearly uniformly throughout the trial.

Secondary endpoint: McGill pain questionnaire

Based on a Wilcoxon signed rank test on the mean changes from baseline, both groups showed statistically significant differences from baseline for all measures. This may have been due to the effect of simply being in a clinical trial. Except for the PPI question, placebo subjects appeared to fare slightly better than their treated counterparts. Based on the t-test on the mean change scores, however, there were no differences between the treatment groups in the changes from baseline.

Pain medication usage is consistent with these results. They are reviewed in the section on tertiary endpoints.

Secondary endpoint: Composite score for change in the GL-3 levels in kidney, skin, and heart

The composite score combined kidney, heart, and skin scores. Because the kidney scores have already been discussed, this briefing document will only discuss heart and skin results here.

Unlike the kidney biopsies, which were subject to special scrutiny because of their place as the primary endpoint, the skin and heart biopsies were not re-read using a detailed quantitative scoring procedure. Three pathologists expert in interpreting the histology of heart and three for skin scored slides from 0-4 based on an overall judgment of severity. A majority score for each slide was determined.

The distribution of baseline severity for heart and skin scores was similar between the treatment groups (Table 53).

Table 13 shows the numbers of subjects with a score of "0" at the end of the trial. The p-value is based upon a χ^2 test. These results corroborate the effect of the product on reducing endothelial substrate levels in kidney.

Table 13. Trial AGAL-1-002-98: Skin and heart biopsy results: Zero and non-zero status in capillary endothelium at the end of the trial*

Organ	End of trial score	Placebo <i>n</i> =29	r-h α Gal <i>n</i> =29
Heart	Zero	1 (3)	21 (72)
	Non-zero	28 (97)	8 (28)
	Odds ratio	0.014	
	p-value	<0.001	
Skin	Zero	1 (3)	29 (100)
	Non-zero	28 (97)	0
	Odds ratio	0.001	
	p-value	<0.001	

*as-treated population, considered equivalent to intent-to-treat

Based on the pathologists' judgments of heart and skin histology, treatment with r-h α Gal reduced heart and skin capillary endothelial GL-3 levels. Quantitative conclusions are hard to make due to the qualitative method of analysis, but levels of reduction in the skin appeared commensurate with those of the kidney, while levels of reduction in the heart capillary endothelium appear to be not as pronounced.

Secondary endpoint: urinary and kidney tissue GL-3

Urinary GL-3 was determined biochemically in filtrates of urine from 24-hour urine collections and in renal biopsy tissue. Table 14 shows the percent change from baseline for urinary GL-3 and kidney tissue GL-3, and the rank sum score derived from these parameters. Genzyme reports a p-value (using a Cochran-Mantel-Haenszel method) of 0.005 for the difference in change between the treatment groups in urinary GL-3 favoring r-h α Gal treatment. The p-value for kidney tissue GL-3 was 0.256, and the combined rank score p-value was 0.003.

The following points are important in review of these data:

- While the renal tissue GL-3 levels sum the substrate from all cell types in the kidney, the urinary GL-3 is thought to derive only from shed renal tubular cells. The precise determinants of the shedding of tubular cells are not known.
- Collection of urine samples for GL-3 was less complete than for the biopsy or pain data. At site 7 baseline urine data were compromised by faulty filtration procedures, and at site 8 an invalid centrifugation method was used; Genzyme omitted data from these sites in their analysis. Genzyme's analysis also omits the data from subject 307; this subject's end-of-treatment sample was lost.
- The median change in urinary GL-3 for the placebo group was considerably positive, an unexpected finding in a placebo group that is expected to be stable. This makes the overall reliability of the urinary GL-3 data suspect.
- Ranks for each subject for each of these determinants were given; the combined score for each subject was obtained as the sum of the ranks of each of the parameters. Since the urinary GL-3 measurement is from cells included in the total renal GL-3 level, the combined score is not based upon entirely separate measurements.
- The reliability of the statistical difference reported by Genzyme for the urinary GL-3 data is compromised by its derivation from a nonrandomized subset of the population. The reliability of the kidney GL-3 statistic is higher given that the great majority of subjects contributed data.

Table 14. Trial AGAL-1-002-98: Urinary and kidney tissue GL-3 and changes from baseline

Parameter	Statistic	Placebo		r-h α Gal	
		baseline	% change	baseline	% change
Urine GL-3 ¹	<i>n</i>	21	21	21	21
	Median	1353	42.8	1612	-23.3
	25 th Percentile	167	-5.7	777	-54.5
	75 th Percentile	3716	420.1	2641	7.5
Kidney Tissue GL-3 ²	<i>n</i>	28	28	27	27
	Median	6510	-6.2	5262	-34.1
	25 th Percentile	3407	-62.1	2326	-79.6
	75 th Percentile	10670	31	9104	19.9
Combined Score of changes	<i>n</i>	21		20	
	Median	48		32.5	
	25 th Percentile	42		24.5	
	75 th Percentile	61		44	

¹ baseline values expressed as nmol/filter

² baseline values expressed as ng/mg

Comments on secondary endpoints

Scores on the McGill pain questionnaire improved in both active and placebo groups during the trial, probably from the psychological effects of being in a clinical trial, but there were no differences between the treatment groups. Other pain-related data (see section on tertiary

endpoints and the section on concomitant medications in the safety review) also failed to show a reduction in pain from treatment with r-h α Gal.

The skin and heart biopsy light microscopy data, obtained in a less rigorous manner than the kidney data, were consistent with the kidney scores in showing that r-h α Gal reduces capillary endothelial substrate levels.

The results for the urinary GL-3 are difficult to interpret for the reasons stated in the description of results for Table 14. The data suggest that the product reduced total renal GL-3 content. The lack of statistical difference between the treatment groups in the measurement of the total GL-3 content in the kidney may be due to a lack of effect in cell types other than the renal interstitial capillaries, as suggested in results from FB9702-01.

Tertiary endpoints

There were 11 protocol-specified exploratory endpoints; these are the primary clinical data from AGAL-1-002-98. The results will be briefly summarized. Some of the measures were assessed in U.S. subjects only (approximately 14-16 subjects); for this population, no dispensing errors occurred and the intent-to-treat population was the same as the as-treated population.

1. Vibration detection threshold, for the U.S. patients only. There was no difference in the change from baseline observed between treatment groups at Visit 11.
2. The Neuropathy Impairment Score, done for U.S. patients only. This is a scoring system based on a routine neurological examination of finger and toe sensation, the cranial nerves, reflexes, and lower limb weakness and reflexes. Overall, there were few differences, with the slight differences observed tending to favor placebo.
3. Neuropathy Symptom and Change Score, done for the U.S. patients only. This is based on subject reporting of their symptoms and the symptoms' changes from a previous period. Changes were similar for both groups for nearly all measures. Genzyme cites the fact that 2 of the 3 autonomic scores (autonomic symptom and severity, not change) as well as the total symptom severity score trended toward greater improvement in treated subjects; however, the baseline scores also were higher in the active group for these measures.
4. The Total Symptom Score (comprised of four symptoms: stabbing pain, burning, prickling/pins and needles, and numbness/feeling of being asleep). Mean scores for both groups fell by less than a point (range of test scores, 0-14.6) in both groups.
5. SF-36 Health Status Survey. This questionnaire was designed to assess general health, not specific for Fabry's disease. It consists of 36 weighted questions falling into 8 categories (physical functioning, role limitations due to physical health, role limitations due to emotional problems, vitality, mental health, social functioning, pain, and general health). Baseline scores were similar for almost all subscales except for role limitations due to physical function; end-of-treatment scores for subscales were almost identical.
6. Physician Assessment of Fabry Symptoms and global perception of subject status. The former consisted of an evaluation of better/not better in terms of angiokeratomas, abnormal sweating, and abdominal pain. There was no between-treatment group difference in the number of subjects rated as getting better in any of these parameters. In the as-treated population, physician answers to the question whether subjects had abnormal sweating changed from "yes" to "no" (baseline to end of trial) in 1 placebo and 3 r-h α Gal-treated subject, a very small difference. Genzyme states that the global perception of subject status (same, improved, or worse) did not differ between the two treatment groups. The investigator's global perception data were apparently not provided in the submission.
7. Number of symptom-free days. This measure trended toward lower values in the r-h α Gal group, with very large variation (as-treated groups: placebo vs. r-h α Gal mean \pm standard deviation, 70 \pm 57 vs. 56 \pm 56).

8. Number of episode-free days (no pain and no medication for pain). This measure trended toward lower values in the placebo group (as-treated groups: placebo vs. r-h α Gal mean \pm standard deviation, 43 ± 53 vs. 48 ± 56).
9. Mean pain score on 0-10 scale. Mean values were slightly different at baseline (as-treated groups: placebo ($n=28$) vs. r-h α Gal ($n=29$) mean \pm standard deviation, 1.9 ± 1.62 vs. 2.2 ± 1.9); for both groups the drop in scores was 0.6 points.

Genzyme presented an additional analysis of diary data not specified in the protocol: number of days in which pain medications were taken, measured in all subjects. This measure trended toward lower values in the r-h α Gal group, with very large variation (as-treated groups: placebo vs. active mean \pm s.dev., 65 ± 63 vs. 58 ± 68). These pain data are difficult to interpret, as the medications were not specified.

10. Glomerular filtration rate, planned for all subjects (see Table 32 in the review of Study AGAL-005, with results included to 18 months of the extension trial AGAL-005-99). The analysis presented by Genzyme must be interpreted with caution: GFR was estimated from creatinine clearance for some subjects and directly by inulin clearance for others. Baseline glomerular filtration rate was not balanced between the treatment groups. GFR improved for the placebo as well as the r-h α Gal-treated group (placebo and r-h α Gal GFRs changed from 97 to 108 ml/min and from 82 to 93 ml/min, respectively), which is a medically unlikely result.

Genzyme has submitted GFR results from baseline of AGAL-1-002-98 to 18 months of AGAL-005-99, calculated with the inulin technique and separately as estimated with an equation that uses plasma creatinine, age, sex, and whether a person is “black”.⁵ These results similarly do not show a benefit of r-h α Gal treatment.

In sum, the data on GFR are not reliable indicators of the true effect, if any, of r-h α Gal on renal function due at least in part to the confounding factors described above.

Genzyme examined serum cystatin C, whose role in the measurement of renal function has not been established clinically as yet. R-h α Gal-treated subjects had a higher baseline value, but values at the end of the trial were similar. These results were inconclusive and may represent a regression toward a mean (nontreated) value.

Comment

CBER examined serum creatinines in AGAL-1-002-98 and AGAL-005-99 (the extension trial) as a measure of renal function. The results show no treatment effect (Table 33, review section of Study AGAL-005).

11. Autonomic status (also termed Global Autonomic Status), done for the U.S. subjects only. It was based on changes from baseline to the end of the trial in the Quantitative Sudomotor Axon Reflex Test (QSART, measuring sweat quantity), the Thermal Detection Threshold just noticeable difference score in the left dorsal foot, and venous occlusion plethysmography (measuring percent volume change in limb blood flow). No reliable clinical difference was shown.

Comments on tertiary endpoints

None of the tertiary endpoints, which were clinically oriented, was solidly positive. The small number of subjects overall, the fact that some of the tests were performed in an even smaller group (U.S. subjects only), the lack of established clinical validity of several of the measurements, and the very brief duration of the trial contributed to the uncertainty of the results. These results do not support a conclusion that galactosidase is ineffective, but neither do they permit concluding that direct clinical efficacy benefits have been established.

“Other” endpoints

1. Ophthalmological examination findings. Genzyme reports that no differences were discerned.
2. Urinary protein to creatinine ratio. Data were only available for two subjects. No analysis was performed by CBER.
3. Renal plasma flow was not assessed due to the unavailability of a reagent used for the test.
4. Plasma GL-3 levels, determined in all subjects. Baseline values were nearly identical (intent-to-treat population: means of 14.7 and 14.4 ng/ μ l in placebo and active groups, respectively); levels at visit 11 showed a notable reduction in the r-h α Gal-treated group (11.0 and 1.3 ng/ μ l respectively, $p < 0.001$).

Total tissue GL-3 levels in skin and heart biopsies as determined by ELISA (not endpoints in the trial). Median percent change were not different between treatment groups (Table 54). However, there was a difference in the change in heart mean and median GL-3 levels at the end of the trial between the two treatment groups. This suggests that there was a reduction due to r-h α Gal in some subjects.

The reliability of the results for skin are questionable; while mean tissue GL-3 for the placebo group did not change appreciably from baseline to end of trial, the median percent change was considerably negative. It would not be expected to change.

The trial included the performance of ECGs and echocardiograms, whose results were not endpoints in the trial. Genzyme reports that there were no changes from the baseline in cardiac conduction and ventricular size. In addition, Genzyme reports no differences between active and treatment groups in echocardiographic determinations.

Comments on “other” endpoints

Plasma GL-3 levels were reduced noticeably in the actively treated population. The clinical relevance of this finding has not been established, but the result is generally supportive of the primary endpoint. Tissue GL-3 levels rendered some minimal support for the primary endpoint in showing some reductions in heart, and possibly skin, levels with treatment.

The ophthalmological findings, like the clinical tertiary endpoints, did not show a clinical change effected by the product.

ECG and echocardiographic data as summarized by Genzyme did not lend support for a benefit of the product.

Antibody development and effect on bioactivity

No subject developed IgE against r-h α Gal. Nearly all of the subjects receiving the Genzyme product (24 out of 29) developed an IgG titer against α -Gal A at some point during the trial (Table 55). The earliest time to development of an anti-r-h α Gal IgG was visit 3, the latest, visit 11.

A subject from the placebo group developed a persistent antibody titer starting at visit 5. The explanation for the event is not clear.

CBER examined the proportions of subjects with scores of “0” at the end of the trial by antibody status. Of the 23 active treatment subjects who seroconverted, 16 (67%) achieved a “0” score at the end of treatment (excluding subject 307, who failed to have a biopsy). Five r-h α Gal-treated subjects did not seroconvert, of whom 4 (80%) had a “0” score at the end of treatment. These proportions suggest that development of antibody during the trial did not have an effect on achievement of the endpoint.

Analyses by CBER failed to show an effect of time to development of antibody (Table 56) or peak titer (Table 57) and the change score on renal histology.

Five treated subjects had non-0 plasma GL-3 levels at the final visit of AGAL-1-002-98 (4/5 were noticeably reduced from baseline of AGAL-1-002-98). The peak antibody titers of these subjects compared to the trial population as a whole did not show a pattern suggesting a correlation of peak titer with non-0 status.

Comment

CBER analyses did not show the effects of antibody development in this brief trial. Results from longer term exposure may suggest that there is little effect of antibody development on the ability to sustain a histological improvement. See results from the extension trial to follow.

Pharmacokinetics

Only 11 r-h α Gal-treated subjects had data analyzed for pharmacokinetics. The conclusions of the pharmacologist were that development of antibodies does not appear to alter terminal elimination half-life (mean 89 ± 20.2 min, range 82–119 min), but it appears to reduce maximal serum concentration and the total exposure. The area under the curve of serum concentration was reduced by the 11th infusion to 27% of initial in a subgroup of 3 subjects with the highest antibody titers ($\geq 12,800$). The maximal concentration fell to 26% in these subjects.

Comment

The drop in exposure due to the development of antibodies raises a concern that histological and potential clinical effects might wane with chronic use in at least a subset of patients taking r-h α Gal.

Summary (bioactivity and efficacy)

The primary endpoint of change to “0” in kidney slide scores was markedly positive, and robust to sensitivity analyses. The studied population was nearly all male, as expected, and nearly homogenous for ethnicity, so subgroup analyses using gender and ethnicity were not feasible. When the results are examined dichotomized at the median age, there is a weak suggestion that the older half of the studied population may have not achieved as much benefit, but this statement is tenuous. Development of antibody to r-h α Gal early in the trial did not limit the extent of reduction at the 5-month endpoint. Reductions in histologically apparent substrate levels were noted in the vascular endothelium of the skin and heart, consistent with the primary endpoint results. However, total GL-3 levels in tissue did not change as consistently, despite a marked reduction in plasma substrate in r-h α Gal-treated subjects.

In response to CBER concerns, Genzyme commissioned the same pathologists who performed the renal histological analysis to perform analyses of additional cell types in the kidney biopsies for this trial. These were done at the same time as these additional biopsies were read as a part of the analysis of the extension trial, AGAL-005-99. Please see the “Bioactivity and efficacy” section of the review of AGAL-005-99 for these important results.

The trial did not show or trend strongly toward any clinical benefit, despite a large number of exploratory clinical analyses.

RESULTS: SAFETY

Similar to the case for measures of efficacy and bioactivity, AGAL-1-002-98 provides the only source of controlled safety data to date. However, this is a brief trial. Longer term, uncontrolled data will be reviewed in this document under the review of AGAL-005-99, the open-label treatment extension to this trial.

Exposure and population analyzed

Twenty-eight subjects received all of their r-h α Gal infusions; subject 503 received 3 infusions of product, and subject 504 received 8 infusions of product.

This analysis of safety excludes subject 503, who only received 3 infusions of r-h α Gal. The omission of subject 503 from the r-h α Gal group would not affect the overall analysis of safety.

Deaths

There were no deaths in the trial.

Adverse events**Serious adverse events**

Nineteen serious adverse events occurred during the trial:

- 8 serious adverse events associated with a biopsy procedure (5 placebo, 3 r-h α Gal)
- 11 other serious adverse event:

placebo:

- accidental injury (fall), convulsions, and speech disorder in a single subject
- worsening of angina, coronary artery occlusion, surgical bleeding, and pericardial effusion in a single subject
- paresthesia
- intracranial hemorrhage (subdural hematoma after a fall)

r-h α Gal:

- worsening of depression in a subject on treatment for depression; this event responded to a change in antidepressant medication. The time of onset of the episode is not detailed in the BLA submission.
- cellulitis. The episode of cellulitis occurred in the ankle of the subject who had a history of osteomyelitis in that ankle, about 1 month after the first infusion. The subject required intravenous antibiotics, and as of this writing of the final report submitted to the BLA had not recovered.

Comment

Serious adverse events were rare in this brief trial. There was no discernible pattern of toxicity of r-h α Gal in these serious adverse event data.

Adverse events: events leading to treatment discontinuation

No subject discontinued participation in the trial.

Nonserious adverse events

Table 58 shows the distribution of adverse events in AGAL-1-002-98.

Rigors (including chills, shaking chills, and cold flashes) and fever were the two events with the greatest increase in frequency and severity in the active-treatment group (Table 58). The great majority (33/40) of adverse events coded as rigors in the active-treatment group were at least possibly due to the infusion, and the majority (9/14) episodes of fever occurred on the day of infusion.

Hypertension as an adverse experience was much less common than in trial FB9702-01. The 3 hypertensive events were associated with infusion reactions (see below).

The term "pain" was reported for more r-h α Gal-treated subjects. It included various events characterized as, for example, "pain," "sore feet," "pains in hands," and "discomfort following a fall." As Table 58 shows, most of these events were mild in severity. "Fabry pain" occurred more often in r-h α Gal-treated subjects, both in mild and in severe events.

CBER did not find evidence for serum sickness in an examination of adverse event terms that might be associated with this syndrome.

Infusion reactions

Infusion reactions were a prominent feature of the administration of the product, occurring in 16/29 r-h α Gal-treated subjects and no placebo-treated subjects. Most infusion-related events were fever and chills, followed by Fabry pain or myalgia. Hypersensitivity reactions, gastrointestinal symptoms, cardiovascular signs and symptoms, and headache accounted for the rest (Table 59).

Suspected hypersensitivity reactions occurred in 12 of these 16 subjects, at the 4th infusion or later. Symptoms in some subjects included chest tightness and shortness of breath, itchiness, flushing, wheezing, and hypotension, as well as the more common shaking chills and fever. (Table 60). These infusion reactions occurred in some subjects despite the institution of steroids in addition to the routine preinfusion medications. With pretreatment the events were mostly of mild to moderate severity but infusion rate adjustments and medications were instituted in most cases. With treatment, infusion reactions resolved. All subjects completed their trial regimen of infusions.

Most, but not all subjects with suspected hypersensitivity reactions had serum IgG to r-h α Gal, consistent with the overall seroconversion rate (Table 61). Although IgE was not tested for every reaction, serum IgE was not found in the great majority of subjects at the last infusion tested, indicating that serum IgE was not required for infusion reactions. The presence or absence of leukocyte α -Gal activity or protein did not correlate with the presence of an infusion reaction.

Comments on nonserious adverse events, including infusion reactions

The most significant adverse event was infusion reaction, which occurred despite pretreatment in many subjects, but resolved with various significant treatments or infusion rate adjustments or both. The presence of immediate hypersensitivity reactions is concerning, despite the fact that subjects continued their treatment regimens in this clinical trial. Data on leukocyte α -Gal, anti- α -Gal IgG, and anti- α -Gal IgE in serum do not allow a prediction of who will experience an infusion reaction.

Hypertension as an adverse event occurred much less frequently during AGAL-1-002-98 than during FB9702-01. The reasons for this are not entirely clear, but could conceivably be due to differences in reporting or to differences in product (AGAL-1-002-98 used a larger scale production method than FB9702-01).

There was no clear indication of immune complex disease, despite seroconversion in the majority of subjects treated with the product.

Genzyme's data do not point to a subject characteristic that will allow prediction of the likelihood of an infusion reaction.

Laboratory abnormalities

Routine hematology, serum chemistries, and urinalysis were determined at baseline and several times during the trial (visits 4, 7, 10, and end of trial). Mean and median values revealed no aggregate treatment-related trend. Laboratory abnormalities listed as adverse events, submitted upon request, did not exhibit concerning patterns. Because of the prevalence of anemia in the Fabry's disease population, CBER examined the proportions of subjects with low hemoglobins at each visit. This analysis revealed no treatment-related trend.

Comment

The data presented on laboratory abnormalities do not suggest a safety concern.

Concomitant medication use

Medication usage data analysis was presented by time period, before and after infusion 1. Although the time period before infusion 1 was not specified, it appears that medication usage for the common symptoms of pain and acroparesthesia was similar in the two groups.

There was an increase in the use of glucocorticoids among the active treated subjects, primarily associated with treatment and pretreatment of infusion reactions.

Antibody development and effect on safety

Comparisons of the frequency of adverse events among subjects with and without antibody was problematic due to the small numbers of subjects in the non-seroconversion group. However, there was no evident difference that correlated with antibody conversion status.

Summary (safety)

The chief concern in this trial was infusion reactions in a large number of subjects. Infusion reactions were significant, being resistant to pretreatment and requiring manipulations of infusion rates and additional treatments. Hypertension, a frequent adverse experience in FB9702-01, was noted only as a component of an infusion reaction, and in a small minority of subjects in this trial. There was no evidence of serum sickness, despite seroconversion in a majority of subjects.

CONCLUSIONS REGARDING AGAL-1-002-98

AGAL-1-002-98 succeeded in its primary objective of showing that r-h α Gal reduces substrate levels in renal capillary endothelium. Although histological analyses for the skin and heart were not as rigorously quantitative as that for the kidney (because they were not based on a method using quantitation of slide parameters), it appears that r-h α Gal had an effect of r-h α Gal on capillary endothelium that is consistent with that shown in the kidney. However, other secondary and tertiary endpoints, including laboratory and clinical measures, did not show a notable treatment benefit. Whether this lack of effect was due to an inability of r-h α Gal to affect critical pathways in the pathogenesis of Fabry's disease, the severity of disease of the subject population, the particular infusion regimen, the brevity of the trial, or some other factor, is an open question.

The chief safety concern was the occurrence of infusion reactions, which were sometimes severe and could occur despite pretreatment medications. With manipulation of infusion regimes and additional administration of medications, subjects were able to continue receiving infusions. There was no clear evidence of antigen/antibody disease despite the prevalence of development of anti-r-h α Gal IgG, and there was no other concerning pattern of toxicity.

Analysis of a small number of subjects suggests that antibody formation can result in a substantial drop in exposure at a given dose. This observation is a concern for a product that is meant to be given for the life of a patient, as it has the potential to signal decreased effect over time.

AGAL-005-99: OPEN-LABEL EXTENSION TO AGAL-1-002-98

After completion of AGAL-1-002-98 subjects were allowed to enter into an open-label extension, AGAL-005-99, entitled “A multicenter, open-label extension study of the safety and efficacy of recombinant human α -Galactosidase A (r-h α GAL) replacement in patients with Fabry disease.”

This trial is ongoing. It is a multicenter, open-label trial in which placebo subjects from AGAL-1-002-98 are placed on active treatment and active subjects from that trial continue on the same treatment. Both groups receive r-h α Gal at the dose and dose frequency chosen for AGAL-1-002-98. Pretreatment for infusion reactions is to be the same as in AGAL-1-002-98, that is, acetaminophen 975 to 1000 mg, and hydroxyzine 25 to 50 mg, orally. The trial's objectives are to determine safety and to ascertain bioactivity primarily by means of kidney, skin, and heart biopsies.

For this document, subjects who originally received placebo in AGAL-1-002-98 are referred to as placebo/r-h α Gal subjects, and those who originally received r-h α Gal in AGAL-1-002-98 are referred to as r-h α Gal/r-h α Gal subjects.

Comment on the design of trial

This trial is most useful to determine whether the histologically observed effect of r-h α Gal on the renal vasculature is a lasting one, especially considering the common development of antibodies to r-h α Gal, and to observe r-h α Gal-treated subjects for safety problems with continued treatment beyond that in AGAL-1-002-98. The group of subjects switched from placebo to r-h α Gal affords a comparison to the r-h α Gal group in AGAL-1-002-98 for objective assessments, allowing a judgment of the strength of the data from that trial on r-h α Gal's effects over 5 months.

Study Design

Subject qualifications

Subjects were to meet the following entry criteria:

Inclusion

- Completion of AGAL-1-002-98

Exclusion

- Having undergone kidney transplantation or being on dialysis
- Clinically significant organic disease with the exception of symptoms relating to Fabry disease

Procedures and evaluations

Subjects were to enroll in AGAL-005-99 upon completion of AGAL-1-002-98. The protocol calls for biopsies of skin at month 6, 12, 30, 42, and 54 (with an optional 18-month biopsy). Biopsy of the kidney and heart are to be performed at month 6 only, but may be postponed to month 12.

The following summarizes other procedures and evaluations in relation to the entry infusion to AGAL-005-99. Final evaluations from AGAL-1-002-98 could serve as entry evaluations if performed within specified time limits.

- Entry and every 2 weeks
 - infusion of r-h α Gal
 - vital signs
 - serum antibody to r-h α Gal (first 24 months only, then every month)
 - adverse event assessment and concomitant medications
- Entry and month 3
 - Physical examination

- McGill short form Pain Questionnaire, SF-36 Health Survey, Fabry Symptom Assessment, Total Symptom Score questionnaires
- Entry and every 6 months up to month 54 except as noted:
 - physical examination
 - ECG
 - echocardiogram at months 6, 18, 30, 42, and 54
 - clinical chemistry and hematology, urinalysis
 - plasma GL-3
 - 24-hour urinary GL-3 up to month 18
 - renal function testing (inulin, PAH, or urea and creatinine clearance)
 - McGill short form Pain Questionnaire, SF-36 Health Survey
 - Fabry Symptom Assessment and Total Symptom Score questionnaires up to month 18
- Pain medication assessment every 3 months beginning month 21
- Neurophysiologic Function test at entry and months 6 and 18
- Ophthalmic examination at entry and month 12
- In the United States only, Gastrointestinal Assessment questionnaire at entry and every 4 weeks up to month 18

Comments

Kidney biopsy, used for the primary endpoint in AGAL-1-002-98, allowed for a determination of the durability of effect. Other evaluations, too, were substantially the same as those in AGAL-1-002-98.

Bioactivity and efficacy measurements

The histological appearance of the kidney was the primary efficacy measurement. In addition, pain, autonomic status, GFR, GL-3 levels, skin and heart histology, urinary protein excretion, ophthalmic changes, vibration detection, and questionnaire data were endpoints.

Analysis of endpoints

Kidney biopsy slides were read by the same renal pathologists, evaluating each capillary to assess a “0” or “non-0” score in the same way as in AGAL-1-002-98 (scores of 1-3 were read using a slightly different algorithm). The pathological analysis of heart and skin biopsy slides was qualitative, as in AGAL-1-002-98, and also by the same pathologists as in AGAL-1-002-98. The analysis of this open-label trial was to be descriptive; in addition, a McNemar’s χ^2 test of matched pairs was to be used to compare proportions of subjects with a score of 0 at entry and month 6 of the trial. Entry scores were the AGAL-1-002-98 end-of-trial scores.

Comments

The trial, despite its open-label nature, allows an assessment of the histological effect among those switched to r-h α Gal, and possible persistence or loss of effect in those continued on r-h α Gal. Its ability to determine clinical effects is severely limited.

RESULTS: DATA SUBMITTED

Data on important outcomes (biopsy and renal function and safety) are available to 18 months. Some data, specified in this document, are available only at 6 or 12 months.

RESULTS: CONDUCT OF TRIAL

Dates of the trial

The trial started in September, 1999 and is ongoing.

Formal protocol modifications

No protocol modifications have been made that would be expected to affect the interpretation of the results.

Enrollment and adherence to infusion schedule and dose

All 58 subjects from AGAL-1-002-98 were enrolled.

The mean and median time to first infusion did not differ notably between the treatment groups.

Adherence to infusion schedule was excellent.

Discontinuations

Twenty-nine subjects started the trial from each treatment group from AGAL-1-002-98 (placebo and r-h α Gal). Dropouts and reasons:

Placebo/r-h α Gal:

Subject 401: after infusion 41, to receive commercial product

Subject 304: after infusion 8, due to skin test reaction to r-h α Gal

Subject 506: after infusion 18, due to death

R-h α Gal/r-h α Gal:

Subject 702: after infusion 41, to receive commercial product

Subject 104: after infusion 23, reason not given

Subject 806: after infusion 36, due to skin test reaction to r-h α Gal

Subject 705: after infusion 52; due to serum IgE to r-h α Gal

Comment

Infusion reactions due to development of IgE are discussed in the safety section for this trial.

Protocol violations

Information on protocol violations is available to CBER through 6 months of AGAL-005-99. Violations of the infusion timing (or cases where infusions were not given) occurred, and there were cases in which biopsies were not performed. However, the frequency of these violations was not sufficient to have a significant impact on the overall conclusions from this trial.

RESULTS: BASELINE CHARACTERISTICS

The subjects in AGAL-005-99 were the same subjects as participated in AGAL-1-002-98.

RESULTS: BIOACTIVITY AND EFFICACY

Results are shown for the as-treated grouping from AGAL-1-002-98, as was done for the AGAL-002 data. It should be noted that data were not available from all subjects at all time points. However, this does not influence the overall conclusions significantly.

Primary endpoint: GL-3 in kidney interstitial capillaries at 6 months

Table 15 shows results expressed as zero score at 6 months (included are two subjects with biopsies postponed to 12 months), separated by treatment group. Most subjects had a score of 0 at

the 6-month time point of AGAL-005-99. One subject (in r-h α Gal continuers) who started with a 0 and ended a non-0, had a non-0 score at 6 months (score of 0 to 1). This subject was identified by each pathologist.

Table 15. AGAL-005-99: Score of "0" on kidney biopsy at 6 months

Treatment Group	AGAL-1-002-98 end of trial score	6-Months of AGAL-005-99		Total
		Zero	Nonzero	
Placebo/r-h α Gal	Zero	0 (0)	0 (0)	0 (0)
	Nonzero	24 (100)	0 (0)	24 (100)
R-h α Gal /r-h α Gal	Zero	17 (94)	1 (6)	18 (75)
	Nonzero	6 (100)	0 (0)	6 (25)

As for AGAL-1-002-98, there was good consistency among the pathologists in the scoring of these slides.

Additional analyses of kidney histology (AGAL-1-002-98 and AGAL-005-99)

In response to CBER's concerns, Genzyme performed analyses of additional cell types from the kidney. These analyses were performed by the same pathologists as performed the analyses in AGAL-1-002-98, but were not based on a quantitative reading of each cell in a biopsy. Table 16 is a summary of the scoring system for each slide, based on an overall judgment:

Table 16. Scoring system for additional cell types (kidney)

Cell/Tissue	Score
Glomerular Endothelial Cells Non-Capillary Smooth Muscle Cells Non-Capillary Endothelial cells	0 = None or Trace Accumulation 1 = Mild Accumulation 2 = Moderate Accumulation 3 = Severe Accumulation
Podocytes DCT/Collecting Ducts	No change Decrease Increase
Mesangial Cells: GL-3 Accumulation Interstitial Cells	0 = No lipid granules 1 = Minimal lipid granules 2 = Numerous lipid granules
Mesangial Matrix	0 = Normal Mesangium 1 = Mild Expansion 2 = Moderate Expansion

Comment

The slides were not fully blinded in this reading. While treatment assignment was coded and blinded, time point was partially known. Baseline slides were identified while the end-of-AGAL-1-002-98 and 6 month of AGAL-005-99 slide time points were not identified as such.

The 6-month kidney results include two 12-month biopsies (postponed from 6 months).

Glomerular capillary endothelium at 6 months

Glomerular capillary endothelium results paralleled those for interstitial capillary endothelium at 6 months, showing the effect of r-h α Gal in reducing substrate levels (Table 17).

Table 17. Glomerular capillary endothelium, proportions of subjects with 0 score at time point among subjects with non-0 baseline score

AGAL-1-002-98 end of trial		6 months of AGAL-005-99	
Placebo	r-h α Gal	Placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
0/16	19/19	21/21	17/17

Podocytes at 6 months

Scores of “severe” were common, and there was little effect of treatment (Table 18). About the same number of subjects had a decrease from baseline at the 6 month time point of AGAL-005-99 (5 in placebo/r-h α Gal and 3 in r-h α Gal/r-h α Gal).

Table 18. Podocytes: Proportions of subjects with no change from baseline*

AGAL-1-002-98				6 months of AGAL-005-99	
Baseline		End of trial			
Placebo	r-hαGal	Placebo	r-hαGal	Placebo/ r-hαGal	r-hαGal/ r-hαGal
Proportion of subjects with “severe” score		Proportions of subjects with no change from baseline of AGAL-1-002-98			
16/16	19/19	16/16	18/19	17/22	14/17

*all subjects had non-0 score at baseline

Mesangial cell matrix at 6 months

There was no notable effect of treatment on the mesangial cell matrix (Table 19).

Table 19. Mesangial cell matrix score (mean \pm std. deviation)

	AGAL-1-002-98				6 months of AGAL-005-99	
	Baseline		End of trial			
	Placebo	r-h α Gal	Placebo	r-h α Gal	Placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
Subjects with data available	21	21	17	21	23	20
Score (mean \pm std. deviation)	0.9 \pm 0.2	0.8 \pm 0.2	0.9 \pm 0.2	0.7 \pm 0.2	0.9 \pm 0.3	0.7 \pm 0.2

Other kidney structuresInterstitial, noncapillary endothelial, and mesangial cells at 6 months

R-h α Gal treatment was associated with a large proportion of scores of 0, even after treatment for about a year total (Table 20).

Table 20. Interstitial, mesangial, and noncapillary endothelial cells, proportions of subjects with 0 score at time point among subjects with non-0 baseline score

	AGAL-1-002-98 End of trial		6 months of AGAL-005-99	
	Placebo	r-h α Gal	Placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
Interstitial cells	0/24	16/23	19/24	23/23
noncapillary endothelial cells	0/22	16/19	19/22	19/20
Mesangial cells	0/16	19/19	19/21	17/17

Noncapillary smooth muscle cells at 6 months

R-h α Gal treatment was associated with a large proportion of decreases from baseline, even after treatment for about a year (Table 21). The amounts of reductions in score were maintained, and possibly increased, with continued treatment with r-h α Gal in AGAL-005-99 (not shown).

Table 21. Noncapillary smooth muscle cells, proportions of subjects with 0 score at time point among subjects with non-0 baseline score

	AGAL-1-002-98 End of trial		6 months of A GAL-005-99	
	Placebo	r-h α Gal	Placebo/ R-h α Gal	r-h α Gal/ r-h α Gal
Proportion of subjects with 0 score	0/22	2/21	0/22	0/21
Proportion of subjects with any decrease in score from baseline	2/22	18/21	19/22	17/21

Distal convoluted tubules and collecting ducts at 6 months

During AGAL-1-002-98 most subjects in both groups experienced no change in score; during AGAL-005-99, decreases outnumbered stable scores and increases in score (Table 22). With continued treatment, more subjects had decreases in score than remained stable or had increases.

Table 22. Distal convoluted tubules and collecting ducts, proportions of subjects with any decrease from baseline*

	AGAL-1-002-98 End of trial		6 months of AGAL-005-99	
	Placebo	r-h α Gal	Placebo/ R-h α Gal	r-h α Gal/ r-h α Gal
Proportions of subjects with any decrease from baseline	1/24	6/24	18/24	12/24

* (all subjects had non-0 score at baseline)

Comment

Reductions in cell types other than renal interstitial capillaries occurred in many, but not all cell types. Despite the lack of full blinding, the results in several cases were quite robust. These results show that the original endpoint results from AGAL-1-002-98 were not a finding isolated to a single cell type.

Endpoint: McGill short form pain questionnaire

Results of a subjective questionnaire during an open label trial are subject to bias. In addition, there was no restriction on the use of pain medications. No remarkable differences

between groups were detected on the McGill short form pain questionnaire (Appendix C: Study AGAL-005-99, Table 62).

Endpoint: GL-3 in skin and heart biopsy

Skin

The pathological analysis of skin biopsy slides was qualitative, on a 0-3 scale.

At 6 months, among the 23 placebo subjects who switched to r-h α Gal in AGAL-005-99 with available data, 21 had a reduction in score to 0 in their superficial capillary endothelial scores, 1 subject had a reduction to a non-0 score, and 1 subject remained at 0.

At 6 months, among the 24 subjects continued on r-h α Gal in AGAL-005-99 with available data, 23 had no change from their 0 score at entry, and 1 had an increase in score.

Table 23 shows skin superficial capillary endothelial scores at 18 months of AGAL-005-99.

Table 23. Skin superficial capillary endothelium (18 months of AGAL-005-99)

Treatment Group	AGAL-1-002-98 end of trial score	18 months of AGAL-005-99		Total
		Zero	Nonzero	
Placebo/r-h α Gal	Zero	1 (100)	0 (0)	1
	Nonzero	19 (90)	2 (10)	21
R-h α Gal/r-h α Gal	Zero	21 (88)	3 (13)	24
	Nonzero	0 (0)	0 (0)	0

Because of concerns over the possible regression exhibited by 5 subjects (non-zero scores at 18 months), Genzyme submitted 30-months results for these 5 subjects (Table 24).

Table 24. Skin superficial capillary endothelial cell substrate scores

Subject	Treatment	AGAL-1-002-98		AGAL-005-99			
		Baseline	End	6 mo	12 mo	18 mo	30 mo
102	placebo/r-h α Gal	2	2	0	0	1	0
107	r-h α Gal /r-h α Gal	2	0	0	0	1	0
201	placebo/r-h α Gal	3	2	0	0	1	0
705	r-h α Gal /r-h α Gal	2	0	0	1	2	0*
806	r-h α Gal /r-h α Gal	2	0	0	0	1	Not done

* approximately 2-year result; subject removed due to detection of serum IgE at 4½ months after 18 month time point

Genzyme points out that the 18-month result for subject 806 was obtained 10 weeks after the last treatment with r-h α Gal. Since the kinetics of possible re-accumulation of substrate are not known, the importance of this observation is unclear.

Comment

The subjects with increased score at 18 months on superficial skin capillary endothelium were among the subjects with the highest anti-r-h α Gal titers. The 30-month results (22 months for subject 705) suggest that the observed increases in scores at 18 months may have been due to chance. Thirty-month results are pending on additional subjects.

Additional cell types in the skin

Genzyme performed analyses of additional cell types from the skin as well. These analyses were performed by the same pathologists as performed the analyses in AGAL-1-002-98. Table 25 shows the scoring system for each slide:

Table 25. AGAL-005-99: Scoring system for additional cell types (skin)

Cell/Tissue	Score
	0 = None or Trace Accumulation 1 = Mild Accumulation 2 = Moderate Accumulation 3 = Severe Accumulation
Deep vessel endothelial cells	
Deep vessel smooth muscle cells	
Perineurium	

Deep vessel endothelial cells

R-h α Gal treatment was associated with a large proportion of decreases in histologically determined substrate in deep vessel endothelial cells, an effect that was maintained at 6 months (Table 26).

Table 26. AGAL-005-99: Skin deep vessel endothelial cells, proportions of subjects with 0 score at time point among subjects with non-0 baseline score

AGAL-1-002-98 End of trial		6 months of AGAL-005-99	
Placebo	r-h α Gal	Placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
1/24	19/23	19/22	23/24

Table 27 shows results at 18 months of AGAL-005-99 among subjects with available data. Six subjects in the r-h α Gal/r-h α Gal group have shown an increase in score from 0 to non-0 at 18 months of AGAL-005-99.

Table 27. AGAL-005-99: Skin deep vessel endothelial cell scores at entry and at 18 months

Treatment Group	AGAL-1-002-98 end of trial score	18 Months of AGAL-005-99	
		Zero	Non-Zero
Placebo/r-h α Gal	Zero	1 (100)	0
	Non-Zero	16 (80)	4 (20)
R-h α Gal/r-h α Gal	Zero	14 (70)	6 (30)
	Non-Zero	2 (100)	0

Comment

Three of the 6 r-h α Gal continuers who showed increased deep vessel endothelial scores at 18 months were among the group with increased superficial skin capillary scores at 18 months. Among the three remaining subjects with increases in deep vessel endothelial scores, 2 were among the entire subject population with the highest maximal titers (1:51200) when determined at any point in AGAL-1-002-98 or AGAL-005-99, and 1 was among the 12 with a maximal titer of 1:12800.

Comments that accompany the endothelial cell biopsy scores suggest that deep structures were sometimes minimal or not available. Genzyme has stated that this could lead to misrepresented analyses. However, the data are consistent with the 18-month data from the superficial capillaries of the skin.

FDA has not received 30-month results for this cell type as of yet, as it has for superficial capillary endothelial cells of the skin, and so has not been able to examine the reductions in scores for their durability at this time point.

Deep vessel smooth muscle cells at 6 and 18 months

Far too little data (1 placebo subject and 6 r-h α Gal subject) are presented to enable any conclusions.

Perineurium at 6 months

There was no notable effect of r-h α Gal treatment in skin perineurium at 6 months in controlled comparison (Table 28). In AGAL-1-002-98, about half of the subjects in both groups remained stable and a small number showed an increase. In AGAL-005-99, about 1/3 of subjects were stable, with 2 increases in score in the former-placebo group and none in the former-r-h α Gal group.

Table 28. AGAL-005-99: Skin perineurium, proportions of subjects with 0 score at time point among subjects with non-0 baseline score

	AGAL-1-002-98 End of trial		6 months of AGAL-005-99	
	Placebo	r-h α Gal	Placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
Proportion of subjects with 0 score	0/22	1/22	1/18	2/20
Proportion of subjects with any decrease in score from baseline	7/22	10/22	11/20	13/21

Table 29 shows results at 18 months of AGAL-005-99 among those with available data. There remained little effect of r-h α Gal on skin perineurium.

Table 29. AGAL-1-002-98 end of trial and AGAL-005-99 18-month skin perineurium results

Treatment Group	AGAL-1-002-98 end of trial score	18 Months of AGAL-005-99	
		Zero	Non-Zero
Placebo/r-h α Gal	Zero	1 (100)	0
	Non-Zero	0	17 (100)
R-h α Gal/r-h α Gal	Zero	0	1 (100)
	Non-Zero	3 (17)	15 (83)

Comments on Additional Skin Cell Type Histology

Skin endothelium, like renal endothelium, responded to treatment with r-h α Gal. However, perineurium did not respond.

Apparent reductions at 18 months in histological effect in skin superficial capillary endothelium for several subjects (Table 23) may have been chance observations. Data on more subjects at 30 months may be helpful in determining the durability of reductions in score.

Heart capillary endothelium

The pathological analysis of heart biopsy slides was qualitative, on a 0-3 scale. Table 30 shows results at baseline and end of AGAL-1-002-98, as well as at 6 months of AGAL-005-99. Sixteen of the r-h α Gal continuers who had an AGAL-005-entry score of 0 maintained that 0 score; 1 of the 22 subjects had an increase in score (not shown); 3 subjects lowered their scores to 0; 2 maintained a non-0 score.

Table 30. Numbers of 0 and non-0 scores in heart capillary endothelium at time points of AGAL-1-002-98 and 6 months of AGAL-005-99

Treatment group	baseline of AGAL-1-002-98		End of AGAL-1-002-98		6 months of AGAL-005-99	
	zero	non-0	zero	non-0	zero	non-0
placebo/r-h α Gal	5	24	1	28	13	5
R-h α Gal/r-h α Gal	5	24	21	8	19	3

Genzyme examined no additional cardiac cell types.

Comment

The results of the cardiac capillary endothelium are consistent with those of other organ endothelial cells in showing a reduction with r-h α Gal treatment. Long-term results are not available.

Endpoint: Urinary GL-3 levels

Analysis for this parameter was only done at the 6-month time point, in 22 subjects in each group (Table 64). There was a decrease in urinary GL-3 in the group switched from placebo to active treatment, and a more complex response among those continued on r-h α Gal (mean and median changing in opposite directions). The interpretation of these data is complicated by the fact that this is a subgroup analysis, with a remarkable amount of variability, making quantitative discriminations between the groups difficult.

Plasma GL-3 levels

Results out to 12 months of AGAL-005-99 were summarized by Genzyme. Plasma GL-3 dropped in placebo subjects switched to r-h α Gal, and remained low in subjects continued on r-h α Gal (Table 31). Four placebo and five r-h α Gal continuers had non-0 plasma GL-3 levels at the month 12 visit (7/9 had appreciable reductions from baseline of AGAL-1-002-98). The peak antibody titers of these subjects compared to the trial population as a whole did not show a pattern suggesting a correlation of peak titer with non-0 status.

Table 31. AGAL-005-99: Plasma GL-3 levels (ng/ μ l) at AGAL-005 entry and at 12 months

Statistic	Placebo/r-h α Gal		r-h α Gal/r-h α Gal	
	Entry	12 months	entry	12 months
<i>n</i>	28	26	28	26
Mean	15.3	0.6	2.3	1.4
Median	10.4	0	0	0
Std. deviation	11.7	1.8	4.32	5.1
Min., Max.	0,41	0, 8	0, 18	0, 26

Comments on plasma and urinary GL-3 observations

The reduction in plasma GL-3 observed with r-h α Gal treatment in AGAL-1-002-98 was seen in the placebo crossovers in AGAL-005-99. Urinary GL-3 data were much more variable and based on a subset of the trial's population. However, they suggest that there is a reduction in GL-3 with treatment with r-h α Gal.

Other endpoints

Renal outcomes, because of their importance to the overall development plan for Genzyme's r-h α Gal, will be discussed first. Data on glomerular filtration rate and serum creatinine include results from the controlled trial AGAL-1-002-98.

- Glomerular filtration rate: Table 32 is extracted from table submitted by Genzyme. For comments on the problems with the interpretation of this table, see comments in the review of tertiary endpoints for AGAL-1-002-98. CBER's analysis out to 6 months using data from inulin technique only yielded similar results, but are on a nonrandomized subset (analysis not shown). Overall, no conclusion can be drawn reliably about the effect of r-h α Gal on GFR from these data. These data do not provide evidence of any improvement with r-h α Gal treatment.

Table 32. GFR (ml/min, mean \pm st. dev.) in AGAL-1-002-98 and at 6 and 18 months of AGAL-005-99

Trial	Visit	Statistic	Treatment group	
			placebo	r-h α Gal
AGAL-11-002-98	Baseline	N	28	29
			97 \pm 35	82 \pm 22
	visit 11	N	23	21
		Mean	108 \pm 39	93 \pm 34
AGAL-005-99	6-months		placebo/ r-h α Gal	r-h α Gal/ r-h α Gal
		N	26	23
	18 months	Mean	117 \pm 41	82 \pm 30
		N	24	26
		Mean	108 \pm 46	80 \pm 30

- Serum creatinine, measured in all subjects. There was no notable effect of r-h α Gal on serum creatinine out to 18 months of treatment (Table 33).

Table 33. AGAL-005-99: Serum creatinine (mg/dl, mean \pm std error)

Treatment Group	Statistic	Baseline AGAL-1-002-98	Entry AGAL-005-99	6 months AGAL-005-99	18 Months AGAL-005-99
Placebo/r-h α Gal	n	29	27	27	27
	Mean	0.8 \pm 0.2	0.8 \pm 0.2	0.9 \pm 0.1	0.9 \pm 0.3
R-h α Gal/r-h α Gal	n	29	27	28	28
	Mean	0.8 \pm 0.2	0.9 \pm 0.2	0.9 \pm 0.04	0.9 \pm 0.3

Genzyme notes that 3 subjects have experienced deteriorations in serum creatinine (1 in the placebo/r-h α Gal group, 2 in the r-h α Gal/r-h α Gal group). Genzyme postulates that these subjects were susceptible to increases due to having “prominent glomerular sclerosis” at baseline. The data are insufficient to draw any conclusion at this time about the mechanism of the deterioration.

- Urinary protein, determined in 20 and 21 subjects in the placebo/r-h α Gal and r-h α Gal/r-h α Gal groups, respectively. Data were not collected at baseline, so do not merit a detailed discussion. However, it is worth noting that urinary protein excretion in this subgroup of subjects was greater at 6 months of AGAL-005-99 in the r-h α Gal/r-h α Gal group than in the group newly switched from placebo (mean \pm std. deviation (mg/24 hr), placebo vs. r-h α Gal, 38 \pm 67 vs. 79 \pm 123; normal is <150 mg/day). Data have not been analyzed at later time points, but this observation merits continued surveillance, as increases in protein excretion may signal increased renal dysfunction.

Comment

The controlled experience with r-h α Gal is very brief, and no effect, beneficial or detrimental was seen. The extension trial AGAL-005-99 was not designed to detect a difference between the groups in renal function, and no effect of r-h α Gal was seen.

- Vibration detection threshold (U.S. subjects only), measured at 6 and 18 months. There was no notable effect of r-h α Gal treatment.
- The Total Symptom Score, measured in the majority of subjects at 6 and 18 months. There was no notable effect of r-h α Gal treatment. The overall interpretation of questionnaire data in an open-label trial is problematic due to the potential for subject bias.
- Gastrointestinal Questionnaire (U.S. subjects only), out to 18 months. The results were not examined. Genzyme states that the data were “unremarkable” for both groups.

- Ophthalmic changes, out to 1 year. The results were not examined. Genzyme states, “No clinically significant changes in any ophthalmic examination parameter were observed for either treatment group between entry and 1 year into the Extension Study.”
- SF-36 Health Status Survey, measured in almost all subjects. During the 6-months of AGAL-005-99, improvements occurred in most subscales for both groups. At 18 months, Genzyme reports that there was a statistically significant change for the placebo/r-h α Gal group between entry to AGAL-005-99 and 18 months on the “physical component” subscale. Due to multiplicity of analysis, the interpretation of this result is problematic; in addition, Genzyme does not comment on the clinical meaning of the changes noted. The overall interpretation of questionnaire data in an open-label trial is problematic due to the potential for subject bias.
- Autonomic status as described in the review of AGAL-1-002-98, assessed in 11 subjects in the U.S. (Table 66). Genzyme states that for the temperature detection threshold and venous occlusion plethysmography neither extension baseline values nor values after 6 months of AGAL-005-99 were abnormal for either group. Genzyme notes that there was a statistical difference seen in thermal detection threshold from entry to 18 months in the r-h α Gal/r-h α Gal group. However, Genzyme does not comment on the clinical significance of this observation, and the clinical significance of other results seen at 18 months. These analyses are limited by the small size of the subgroup in which they were tested; the importance of statistical tests is limited by the fact that multiple tests were applied to these data, without specification of the relative importance in advance.
- Physician assessment of Fabry symptoms. There were no notable changes in numbers of subjects with changes in scores (yes or no) on questions relating to abnormal sweating or abdominal pain. Slightly more subjects had abdominal pain at 18 months than had it at entry in both groups (Table 67).

Summary comments on bioactivity and efficacy from AGAL-005-99

Histological analyses showed that reductions of substrate from capillary endothelium of kidney, skin, and heart were sustained in subjects continuing to receive r-h α Gal, and that the reduction seen in the r-h α Gal group from AGAL-002-98 was reproduced in placebo crossovers during the 1st 6 months of subsequent open-label treatment. Preliminary suggestions that some subjects had experienced regression in effect may have been due to chance observations. Further data may help to determine the durability of reductions in scores.

Clinical endpoints, as well as measures of renal function, show no effect of treatment.

RESULTS: SAFETY

The following is a discussion of the safety record of the AGAL-005-99. Data have been submitted up to infusion 42.

Deaths

There has been one death reported up to infusion 42 of AGAL-005-99. This was a 43 year-old man who had been on placebo during AGAL-1-002-98, who suffered a cardiac arrest and dysrhythmia 400 days after being on r-h α Gal. His medical history is stated as “severe heart disease associated with marked acute heart failure and findings consistent with Fabry disease.”

Adverse events

Serious adverse events

Twenty-three of the 58 subjects experienced serious adverse events. Counting terms that were related to each other that occurred on the same day as the same event, the serious adverse events may be grouped as follows:

- Related to biopsy: 3 subjects, 3 events
- Related to infusion: 6 subjects, 7 events. As Table 34 shows, serious infusion-related events may occur late in treatment.

Table 34. Infusion-associated serious adverse events in AGAL-005-99

subject	term	days after treatment
201	tachycardia	32
	hypertension	33
304	pruritic urticaria	98
306	tightness in chest	55
	tightness in throat	55
705	swelling and erythema in ear, warmth in face	881*
805	fever	128
	very intense shivering	128
	tachycardia	128
806	abdominal pain	582
	cutaneous rash	
	skin redness	
	vomiting	
	pruritis	
	nausea	
	shivering	

*post-18-month data

- Cardiac/neurologic events: 6 subjects, 7 events. Serious cardiac and neurologic events occurred mostly several to many months after the first infusion of r-h α Gal (Table 35). Two strokes occurred in subjects with no prior history, but stroke is a known complication of Fabry's disease. Subject 603 had an episode of chest pain during this trial, with no documented cardiac abnormality. An additional, ischemic event occurred: hand ischemia in a 41 year-old subject (subject 708) 175 days after the start of treatment, in a subject with no relevant medical history.

Table 35. Serious cardiac and neurologic adverse events in AGAL-005-99*

Subject	Term	Age	Days after treatment	Relevant history
109	Stroke	37	770	no
112	Stroke	25	870	no
501	Stroke	42	611	yes
502	Worsened angina	62	53	yes
506	Bradycardia	43	242	yes
	Decreased cardiac output		242	
	Cardiac arrest**		400	
	Dysrhythmia**		400	

* See text for additional ischemic/possibly ischemic events

**reported as death

- Miscellaneous: See Table 36.

Table 36. AGAL-005-99: Miscellaneous serious adverse events (one row per subject)

Event	Medical history
Viral pericarditis	none related
Hand injury	none related
Suicide attempt	none related
Head and hand injury	none related
Bronchitis	asthma, tobacco use
Vertigo (twice) and hypoacusia	vestibulocochlear disorders, tinnitus, bilateral hearing loss, vertigo
Carpal tunnel syndrome	none related
Herpes labialis of leg with erysipelas	none related
Suicide attempt	none related
Basal cell carcinoma	none related
Basal cell carcinoma	No cancer related history
Nephrotic syndrome	Fabry related-proteinuria, extensive baseline glomerular scarring
Loss of visual acuity, macular edema, retinal white dots syndrome	inflammatory syndrome
First metatarsal osteitis	cutaneous wound ulcer

Comments

The group switched to r-h α Gal experienced several serious cardiac adverse events, but serious adverse events of this nature did not occur in the r-h α Gal-treated group in AGAL-1-002-98, so they do not constitute a strong pattern. There is no clear pattern of serious events other than those associated with biopsy and infusion.

Infusion reactions

During the first 6 months of AGAL-005-99

The majority of subjects experienced infusion reactions in the first 6 months of AGAL-005-99. Table 37 shows the numbers of subjects with any adverse event in groups of infusion-related syndromes.

Table 37. AGAL-005-99: Subjects with infusion-related adverse events at 6 months

Adverse Events	Subjects with any infusion-related event <i>n</i> =34
Febrile reactions: fever, feels warm, feels cold, or chills	30
Hypersensitivity: dyspnea, throat tightness, flushing, chest tightness, pruritis, urticaria, or rhinitis, bronchial constriction, tachypnea, or wheezing	17
Pain symptoms: Fabry pain or myalgia	8
Gastrointestinal: abdominal pain, nausea, or vomiting	8
Cardiovascular: tachycardia, palpitations, or hypertension	6
Headache	6
Fatigue and related symptoms	5
Edema	4

In addition to these events, the following events categorized as “severe” occurred on the day of infusion.

- Subject 501 (r-h α Gal/r-h α Gal) experienced severe shivering
- Subject 505 (placebo/r-h α Gal) experienced severe pains in both hands
- Subject 804 (r-h α Gal/r-h α Gal) experienced severe shivering and skin edema
- Subject 805 (placebo/r-h α Gal) experienced severe urticaria

Treatment consisted of a reduction in infusion rate, with various combinations of antihistamines, inhaled beta agonists, NSAIDs, or steroids.

More infusion-related events that were suspected to be due to hypersensitivity occurred in subjects crossed over to r-h α Gal treatment from placebo during AGAL-005-99 (15/25) than in continuers during the first 6 months of AGAL-005-99 (Table 65). On the other hand, over half of subjects (7/12) who had had infusion reactions while on r-h α Gal treatment during AGAL-1-002-98 had them in AGAL-005-99. Serum IgG antibody was detected at the time of nearly all infusions; testing for complement activation was sporadic.

Trends relating to infusion reactions during the first 18 months of AGAL-005-99

CBER examined summaries of adverse events classified as related to treatment that occurred on the same day as infusion, divided by approximate 6-month time periods: baseline to visit 11 (5 months) of AGAL-1-002-98, entry to AGAL-005-99 to 6 months of the trial, 28 weeks to 1 year after entry to AGAL-005-99, and 54 weeks to 18 months after entry to AGAL-005-99. The incidence of infusion-related events generally decreases over time, and there is no event with a reliable increase in incidence. In the period from about 18-24 months after initiation of treatment (subjects on r-h α Gal in AGAL-1-002-98), 4 subjects had infusion-associated nausea and 2 subjects each had the following events: rigors, hypertension, and vomiting. One subject each had paresthesia, abdominal pain, tachycardia, arthrosis, rash, ECG abnormal, hypotension, skin discoloration, anxiety, cardiac failure, hypertonia, and myalgia (the same subject may have had more than one event).

IgE-mediated infusion reactions/ events leading to withdrawal

With regards to the occurrence of infusion reactions that would prompt testing for IgE, the following summary of the testing of serum samples for IgE up to month 12 months of AGAL-005-99 is relevant:

- 12/29 (41%) r-h α Gal subjects in AGAL-1-002-98 had serum tested for IgE (all negative)
- 18/58 (31%, all r-h α Gal) in the 1st 6 months of AGAL-005-99 were tested for IgE (all negative)
- 3/58 (all r-h α Gal) in the 2nd 6 months of AGAL-005-99 were tested for IgE (all negative)

In trial AGAL-005-99 several subjects were withdrawn for evidence of development of IgE to r-h α Gal (see tabulation in the section on discontinuations). It is important to note that these reactions could occur out to infusion 52 of the trial.

Comment

Infusion reactions to r-h α Gal decrease in incidence with time. While there is evidence that suspicion for IgE-mediation for infusion reactions may go down with time, it is important to note that the development of serum IgE has been seen out to infusion 52 of an every-other-week infusion schedule. Because nearly all subjects were IgG antibody (+) during the trial (see below), testing for IgG antibody would not allow determination of subjects at risk for development of infusion reactions.

Other adverse events and laboratory abnormalities

There has been no increase in particular adverse events in the continuers on r-h α Gal compared to those who were switched from placebo to r-h α Gal (Table 68). Severities of events are

generally similar (not shown). Adverse events have been generally consistent with the course of Fabry's disease. There has been no remarkable laboratory toxicity to date.

IgG antibody to r-h α Gal

Data were available up to infusion 41 of AGAL-005-99. The majority of subjects in the placebo/r-h α Gal group seroconverted (25/28, with one additional subject from the placebo group in AGAL-1-002-98 remaining seropositive). Among the subjects who remained on r-h α Gal from AGAL-1-002-98, 27/29 seroconverted at some point before or at infusion 41, and 26/29 subjects were seropositive at infusion 41 (with 1 missing observation).

Comment

CBER has requested that Genzyme explore possible correlations of measures of the extent of antibody development with levels of reduction in substrate or possible regression in histological score) in AGAL-005-99.

CONCLUSIONS REGARDING AGAL-005-99

The results of AGAL-005-99 support the conclusion from AGAL-1-002-98 that r-h α Gal causes a reduction in GL-3 deposition in capillary endothelium of the kidney, heart, and skin. Histological reductions in substrate persisted for most subjects, but there was some evidence that with prolonged treatment the reductions might wane.

Nearly everyone exposed to r-h α Gal has developed IgG antibody to galactosidase.

There were no additional clinical findings or findings from other laboratory evaluations that revealed a clinical benefit.

Infusion reactions waned with time, but late development of problematic IgE reactions to the product remain a concern.

AGAL-007-99

Genzyme conducted a trial in Japan that was not conducted under IND or reviewed by CBRE prior to its initiation. The trial, AGAL-007-99, was entitled "A Multicenter, open-label study of the safety and efficacy of recombinant human α -Galactosidase (r-h α GAL) replacement in patients with Fabry Disease.

The findings of this trial have not been subject to detailed scrutiny, but were reviewed to discern any remarkable differences in efficacy or safety trends. A brief summary of the findings is presented here.

AGAL-007-99 was an open-label 13-subject, 5-month trial of r-h α Gal at the same dose and frequency as studied in AGAL-1-002-98 and AGAL-005-99. Subject qualifications were the same as in AGAL-1-002-98. While the trial was open-label, evaluation of the primary endpoint, renal histology, was done on fully blinded slides by the same pathologists and using the same quantitative scoring method as used for the primary endpoint analysis of AGAL-1-002-98 and AGAL-005-99. Nonquantitative methods were used for skin and heart, consistent with AGAL-1-002-98 (heart biopsies were to be performed only on those patients with cardiac abnormalities demonstrated at baseline). Many of the same clinical outcome measures were also assessed. Accumulation of substrate in additional cell types was also assessed.

Fourteen subjects were screened; 13 met eligibility criteria and enrolled; all completed all 11 infusions. The subjects had a slightly lower weight and age than those enrolled in AGAL-1-002-98, and they were all males. Other baseline characteristics were generally similar.

There were no protocol deviations that would be expected to have a major impact on determinations of safety and efficacy.

Bioactivity and efficacy resultsHistology

- Kidney capillary endothelium: 10 subjects started with mild substrate accumulation; 3 with moderate. All ended with a score of 0 except for one subject with moderate accumulation at baseline, whose score was 1 (mild) at the end of the trial.
- Additional renal cell types: Due to some biopsy samples not containing the cell type of interest, sample numbers were not 13 in all cases for all time points.
 - Glomerular endothelial cells. Paired results for 11 subjects: 7 subjects started with severe scores, and 4 with moderate scores; all ended with 0 scores.
 - Kidney non-capillary interstitial endothelial cells. Paired results for 12 subjects: 10 subjects started with severe scores, and 2 with moderate scores; all ended with 0 scores.
 - Kidney non-capillary interstitial smooth muscle cells. Paired results for 12 subjects: 8 subjects changed from severe to moderate, and 4 changed from severe to mild.
 - Podocytes. Paired results for 11 subjects: 9 subjects stayed severe, and 2 changed from severe to moderate.
 - Kidney distal convoluted tubules and collecting ducts. 13 paired samples: 10 showed reductions in score.
 - Mesangial cell matrix of the kidney. There were 11 samples at baseline and 12 at the end of the trial. Mean and median values were unchanged.
 - Mesangial cells of the kidney. Paired results for 11 subjects: all subjects started with a score of numerous lipid granules: 2 subjects changed to minimal, and 9 changed to no lipid.
 - Interstitial cells of the kidney. Paired results for 13 subjects: All subjects started with a score of numerous lipid granules: 1 subject was unchanged, 2 subjects changed from numerous lipid granules to minimal, and 10 changed from numerous lipid granules to no lipid granules.
- Skin cells
 - Capillary endothelium. Thirteen paired samples, all with reductions.
 - Deep vessel endothelial cells. 12 paired samples, all with reductions.
 - Smooth muscle cells. No paired samples were available.
 - Perineurium. 12 paired samples, 4 with reductions.
- Heart capillary endothelium: Only 1 subject had heart biopsies at baseline and end of trial. Cardiac capillary endothelial cell accumulation changed from mild to none.

Laboratory findings

- Creatinine clearance (ml/min). Based on 13 samples at baseline and the end of the trial, there was a non-significant decline in mean creatinine clearance (126.6 ± 42 to 115 ± 30). The method of determination of this result is not presented.
- Serum creatinine. Mean serum creatinine did not change from baseline to the last visit.
- Plasma GL-3 (ng/ μ l). Based on 13 samples at each time point, the mean value fell from 3.9 ± 2.7 at baseline to 0.2 ± 0.8 at 11 weeks.

Clinical findings

- Angiokeratomata, abnormal sweating, and abdominal pain: There was no significant change from baseline.

Safety

There were no deaths in this trial. There were two serious adverse events: 1) fever, limb pain, malaise, and congestion of the nose starting at an infusion 112 days after the start of treatment, and 2) infectious gastroenteritis, severe limb pain, and a (+) reaction for C-reactive protein 69-70 days after the start of treatment.

Infusion reactions were common in AGAL-007-99. Ten of 13 subjects experienced reactions on the day of infusion that included events such as fever, nasal congestion, cough, rigors, limb pain, and dyspnea.

Seven subjects had infusion reactions that raised the suspicion of relation to development of IgE. These reactions occurred at infusions 4-10. Serum IgE against r-h α Gal was not detected in any subject; skin testing was not performed.

The most common nonserious adverse events were:

- Albuminuria. Eleven subjects had albuminuria; 10 of the events were mild. Seven of the 11 subjects had a history of proteinuria
- Bradycardia. Nine subjects, all with mild bradycardia. Three subjects had a history of bradycardia. The reason for bradycardia was not clarified in the submission, but it was noted that manual recording of pulse rate was performed during infusions.
- Rhinitis: Eight subjects had mild rhinitis during the trial.

There was no notable effect of r-h α Gal on means of serum chemistries or hematologies at baseline and end of the trial.

Seroconversion

Eleven of 13 subjects seroconverted at a mean time of 63 days (range, 26 to 126 days).

Comments

Histology results were consistent with results seen in AGAL-1-002-98. There was no clinical efficacy, and no notable effect on renal function. The safety results are consistent with those of the American/European trials.

ADDITIONAL SAFETY INFORMATION

The following section summarizes additional serious adverse event and death information received from Genzyme related to additional trials, through February 28, 2002:

- Ongoing open-label extension study AGAL-006-99:1 mg/kg (15 subjects), with duration of exposure not shown.
- Ongoing randomized, double-blind, placebo-controlled trial AGAL-008-00 (over 50 subjects), with duration of exposure not shown.
- Special access and other programs in the U.S. and Europe (number of subjects not specified).
- Postmarketing experience. The number of patients is not shown (there are approximately 176 patients worldwide who receive Genzyme's commercial r-h α Gal as of August 14, 2002).

Deaths

Table 38 shows information related to deaths.

Table 38. Deaths in trials other than AGAL-005-99 to February 28, 2002

Study/ Patient	Age/ Sex	Treatment group/ Dose q14d	Date of First Infusion/ Date of Onset/ Date of Death	Duration of R-h α Gal treatment before SAE (days)	Verbatim Term	Medical History
AGAL-008-00/ 18041	51/M	Placebo/ R-h α Gal 1.0 mg/kg (Blinded)	03OCT2001/ 30OCT2001/ 30OCT2001/	25 (blinded)	Cardiac arrest	Hyperlipidemia, gout, and kidney stones and findings consistent with history of Fabry disease
AGAL-008-00/ 12041	55/M	Placebo/ R-h α Gal 1.0 mg/kg (Blinded)	05FEB2002/ 06FEB2002/ 13-Feb-02	2 (blinded)	Stroke	Ischemic heart disease, decreased left ventricular function, and renal impairment
US Single Patient Exemption AGAL-010-00/ Single patient	59/M	R-h α Gal 1.0 mg/kg	18SEP2000/ 18SEP2000/ 24-Sep-00	1	Anasarca	Severe Fabry disease associated with severe renal, cardiac and pulmonary involvement; also central nervous system involvement with a past cerebrovascular accident and residual hemiparesis.
				UNK	Sepsis	
Europe Treatment Use (Netherlands)	48/M	R-h α Gal 1.0 mg/kg	05APR2001/ 24APR2001/ 24-Apr-01	5	Cardiac Arrest	Four myocardial infarctions (1986, 1989, 1997, 1998).
Japan Treatment Use/ AGAL-011-00	63/M	R-h α Gal 1.0 mg/kg	28DEC2000/ 16SEP2001/ 16-Sep-01	263	Ventricular Tachycardia	Extensive cardiac history including sick sinus syndrome, left ventricular hypertrophy, and congestive heart failure
Europe Treatment Use (under French ATU)	53/M	R-h α Gal 1.0 mg/kg	06JUN2001/ 18JUL2001/ 18-Jul-01	42	Ischemic colitis, Multiple organ failure	Hemodialysis 3 times a week, long history of abdominal pain

*There was one death noted in the review of AGAL-005-99.

Comment

Most of the deaths, including the death in AGAL-005-99, are consistent with vasculopathy, and possibly with the natural course of Fabry's disease; three of these occurred at or within 6 weeks of the start of treatment. As two of these three events were blinded, the evidence implicating treatment is weak, but is cause for being watchful.

Serious adverse events (excluding deaths)**Trial AGAL-006-99**

Five subjects had serious adverse events. Times to event do not precisely reflect time from the start of treatment, since subjects had spent variable times on r-h α Gal during the core trial, FB9702-01. These events are of miscellaneous natures. Detailed information is available only for the last event, as the others were not deemed related to treatment, and the last was deemed possibly related.

- 1) vomiting in a subject with a history of irritable bowel (at 8 days of treatment)
- 2) atrial fibrillation in a subject with a history of junctional rhythm and SVT (at 76 days of treatment)
- 3) uvula edema in a subject with family member with a "similar condition" (at 92 days of treatment)
- 4) perforated diverticulitis and peritonitis in a subject with a history of cramping and diarrhea (at 1242 days of treatment)
- 5) chest pain, fatigue, and dyspnea in a subject with a history of cardiac disease and pacemaker (at 32 days of treatment). The subject was discontinued from treatment.

AGAL-008-00

The events from this trial are blinded.

Thirteen subjects experienced serious adverse events after initiating treatment. Counting terms related to each other that occurred on the same day as the same event, the serious adverse events may be grouped as follows:

- "Probable" relationship to infusion: 1 subject, 1 event: angioedema, flushing, wheezing, urticaria, and cough 43 days after start of treatment.
- "Definite" relationship to infusion: 1 subject, 1 event: hypotension, at 54 days of treatment (infusion 5). This subject had IgE to r-h α Gal and was withdrawn from the trial.
- Miscellaneous: 4 subjects, 5 events:
 - an acute attack of Meniere's syndrome in a subject with history of stroke, angina
 - syncope in a subject with asthma
 - mood disorder and medication adjustment in subject with post-traumatic stress disorder and depression
 - fever in subject with "similar febrile reactions, although not in the past year"
- Possibly cardiac/neurologic: 6 subjects, 6 events (Table 39). Note that narratives are not available to discern details of these cases, as the events were not deemed to be due to treatment.

Table 39. Serious possible or diagnosed cardiac and neurologic adverse events in AGAL-008-00 (blinded treatment)

Subject	Term	Age	Days after treatment	History
20041	Chest pain	36	40	Cardiac disease
20047	Atrial fibrillation	52	68	Pacemaker
24041	Chest pain	54	105	Cardiac disease
27041	Stroke	59	42	Cardiac disease
34042	Auricular disease	54	43	Cardiovascular; hypertension, left AV

				bundle branch block
12041	Stroke	55	2	Ischemic heart disease; decrease LV function; and renal impairment

*Narratives not available to determine details of the diagnoses and treatments

Comments

Since submission of the safety report upon which the summary of safety in the additional trials is based, Genzyme has submitted a report of the development of a (+) skin test, rash, shakes, headache, fever, and urticaria in another subject in AGAL-008-00. This subject met protocol-specified criteria for withdrawal.

Importantly, this trial allows enrollment of subjects with potentially moderately advanced renal disease, which may also include more advancement of cardiac and neurological manifestations of their disease. This may account for the large number of relatively early cardiac and neurological events.

Serious adverse events from special access programs

Subject characteristics from these programs are not presented, and details are not available for all events.

Three subjects had 5 miscellaneous serious adverse events:

- anemia, elevated C-reactive protein, and ileus in a subject with end-stage renal disease and cardiovascular disease
- musculoskeletal pain in the upper abdomen/chest in a subject with no related medical history
- rhabdomyolysis; subject noted to have had red discoloration of the urine thought due to rifampicin (not clear if applicant's submission describes event or medical history)

Possible or diagnosed cardiac or neurological events are shown in Table 40. All but the stroke were thought possibly (---- and ----) or probably (-----) to be due to r-h α Gal.

Table 40. Serious possible or diagnosed cardiac and neurologic adverse events in special access programs

Subject	Term	Age	Days after treatment	Relevant history
-----	Myocardial infarction	36	Unknown	Mild chest pain
-----	Ataxia; urosepsis, disorientation	44	6	Cerebellar stroke
-----	Stroke	44	264	3 left hemispheric strokes
-----	Retrosternal chest pain**	63	170	Cardiomyopathy, stroke, peripheral vascular disease

*Details not available in submission

**Responded to nitroglycerin, but not diagnosed conclusively

Post-marketing serious adverse events

Three serious adverse events are presented, all in subjects on the proposed dose. Only the last was deemed due to treatment.

- 1) Toe infection in a 41 year-old subject with no relevant medical history, 121 days after the start of treatment
- 2) Umbilical hernia in a 47 year-old subject with a history of peritoneal dialysis, 71 days after start of treatment
- 3) Infusion-related reduced blood pressure, increased sweating, bronchospasm, and somnolence in a 14 year-old subject 43 days after the start of treatment.

Summary comments on additional safety data

The largest single group of events were possibly vascular: cardiac and neurological events. Some of these events occurred shortly after treatment. However, because of the lack of a control group, the predisposition of patients with Fabry's disease to vascular events, and the documented

history of cardiac and neurological events in some of the subjects, there is not a strong safety concern at this time.

Infusion-related events were consistent with those in the clinical trial data presented earlier, and merit continued concern.

FINANCIAL DISCLOSURE

There were two investigators with relationships to Genzyme that merited scrutiny. Both were listed as subinvestigators on AGAL-1-002-98, the primary source of efficacy information. Dr. Robert Desnick, who was central to the development of Fabrazyme, and who is listed in patents involving Fabrazyme, was a subinvestigator at the Mt. Sinai Medical Center. Dr. Hans Aerts, was a subinvestigator at the Academisch Medisch Centrum. CBER's review of the financial relationship of Dr. Desnick and Dr. Aerts, and of the roles they played in the trial, show that there is no significant concern over bias in the results of the trial.

OVERALL SUMMARY OF BIOACTIVITY AND EFFICACY

The clinical data presented provide for the following conclusions:

- Intravenous administration of Genzyme's r-h α Gal results in reduction of the enzyme's substrate in capillary endothelial and selected other cells of the kidney, heart, and skin. However, the effect of the product on nonvascular cell types and tissues is variable, and sometimes negligible to nonexistent. The pathophysiology of Fabry's disease does suggest that reduction of endothelium has the potential to decrease the microvascular pathology of the disease, however, so it may be reasonable to judge that the product could show clinical benefit.
- There is pharmacologic evidence of a reduction in exposure to r-h α Gal in individuals with high-titer antibody. These data open the question of the likelihood of continued effect in these individuals. More long-term skin biopsy data, up to now available in a limited number of subjects, may provide further insights into the histological effects of r-h α Gal with chronic administration.
- The clinical trials failed to show clinical benefit on a wide range of tests of neurologic, renal, and cardiac function. The design of these studies was not with objective of showing clinical efficacy, however. Whether a longer trial, or one with different eligibility criteria, could have demonstrated a benefit is an open question.

OVERALL SUMMARY OF SAFETY

- The chief safety problem with the administration of Genzyme's r-h α Gal was the occurrence of infusion reactions. Reactions have required adjustment of infusion rates, administration of systemic corticosteroids and other medications, and have occurred despite premedication with nonsteroidal anti-inflammatory medications and antihistamines; and 5 subjects have met protocol requirements for withdrawal due to detection of anti-r-h α Gal IgE. Genzyme knows of no predisposing factors to the development of infusion reactions.
- Noninfusion-related toxicities appeared to be minimal, but the ability to discern toxicities related to chronic treatment in an ill population is diminished by the open-label nature of the data.
- Antibody formation was extremely common, and persistent. The continued presence of antibodies poses a potential safety risk for the development of continued infusion reactions.

ONGOING CLINICAL STUDIES

Besides the ongoing open label extension studies, Genzyme has Study AGAL-008 in progress. AGAL-008 is double blind, placebo controlled study in approximately 30 centers enrolling approximately 70 subjects and randomizing 2:1 r-h α Gal to placebo. The dose of r-h α Gal is the standard 1 mg/kg IV dose. Treatment continues for 35 months or until the prospectively determined required number events for statistical powering occurs. Interim analyses of the aggregate data will determine the actual study duration. The primary objective of this study is to demonstrate the clinical efficacy of r-h α Gal in preventing meaningful adverse events or adverse progression of the disease in the renal, cardiac, or neurological systems.

Eligibility Criteria:

Inclusion Criteria:

- 16 years old or greater
- Diagnosis of Fabry Disease and consistent clinical presentation
- No prior receipt of enzyme replacement treatments
- Plasma α -Gal activity < 1.5 nmol/hr/ml or leukocyte α Gal < 4 nmol/hr/mg
- Evidence of renal impairment at baseline:
 - 2 consecutive serum creatinine between 1.2 and 3, with less than 15% difference or
 - creatinine clearance estimate of < 80 ml/min using Cockcroft-Gault formula with less than 15% difference between 2 measurements

Major Exclusion Criteria;

- Renal transplant received or scheduled
- Acute renal failure
- Stroke or TIA within 3 prior months
- Advanced cardiac disease (explicit criteria provided)

Major evaluations in the study include serum creatinine every 4 weeks, with a broader range of assessments every 3 months.

Primary Endpoint:

Time-to-event for any of the “events” defined in the renal, cardiac, or neurologic systems. Renal events are expected to be the most common, and consist of an increase in serum creatinine by 33% from baseline or the need for dialysis for more than 40 days. Cardiac events include MI, new symptomatic arrhythmia, unstable angina, new or worsening heart failure. Neurologic events include new stroke or TIA.

Secondary Endpoints include an analysis of the slope of change in creatinine, time to renal (only) progression, pain score, and the slope of change in estimated GFR. GFR is estimated from age, weight, and serum creatinine. No direct measure of GFR is performed.

CONCLUSIONS AND CURRENT STATE OF CLINICAL ACTIVITY

Based on the premise that Fabry's disease is primarily a vascular disorder, Genzyme proposes that the histological data are sufficient to conclude that there is a reasonable likelihood that r-h α Gal will confer a clinical benefit. However, no robust clinical benefit has been shown. The reasons for this may be due to inherent lack of connection between histological bioactivity and effect or an inability of the trials as designed to detect clinical differences.

Genzyme has requested that they receive Accelerated Approval for their galactosidase product. Genzyme is currently conducting a double-blind, randomized, placebo-controlled trial intended to determine if their r-h α Gal will confer a clinical benefit in moderately severe patients with Fabry's disease (AGAL-008-00). If they receive an accelerated approval for r-h α Gal, the ability of AGAL-008-00 to conclusively determine the clinical effect of r-h α Gal would become critical. This trial is currently nearly fully enrolled. Genzyme currently has a proposal to convert this trial into an open-label, single-arm trial, with a comparator consisting of data from a natural historical data base. The review of this database is the subject of another briefing document.

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APPENDICES (TABLES REFERRED TO IN TEXT)

Appendix A: Study FB9702-01

Table 41. Trial FB9702-01: Demographics and baseline characteristics

	0.3/14-day <i>n</i> =3	1.0/14-day <i>n</i> =3	3.0/14-day <i>n</i> =3	1.0/48-hr <i>n</i> =3	3.0/48-hr <i>n</i> =3
Age (yr) mean \pm std error Series	41.0 \pm 3.0 35, 44, 44	33.7 \pm 3.4 27, 36, 38	34.7 \pm 1.5 32, 37, 35	27.0 \pm 5.5 37, 26, 18	35.7 \pm 4.7 45, 30, 32
Weight (kg) mean \pm std error Series	64.7 \pm 4.1 66, 57, 71	73.6 \pm 9.1 88, 57, 76	69.1 \pm 7.4 56, 82, 69	69.8 \pm 2.4 73, 65, 72	78.0 \pm 8.0 74, 67, 93
Gender (<i>n</i>) male	3	3	3	3	3
Race (<i>n</i>) White Black Hispanic	3 0 0	1 0 2	2 0 1	2 0 1	3 0 0
Serum creatinine Mean \pm std error Series	1.3 \pm 0.4 0.6, 1.3, 2.0	1.2 \pm 0.1 1.4, 1.0, 1.1	1.4 \pm 0.2 1.5, 1.6, 1.1	1.0 \pm 0.3 1.6, 0.8, 0.6	1.6 \pm 0.2 1.9, 1.7, 1.1
Plasma α -gal	BDL*	BDL*	BDL*	BDL*	BDL*
Prestudy plasma GL-3 (ng/ml) mean \pm std Series	22.0 \pm 3.1 16.6, 27.4, 22.1	15.2 \pm 4.3 16.7, 21.7, 7.2	29.5 \pm 10.7 48.6, 28.2, 11.6	20.0 \pm 3.2 20.3, 14.3, 25.5	3.3 \pm 1.6 6.2, 2.6**, 1.0**

*below 55 ng/ μ l

**below the inclusionary limit of \geq 5 ng/ml

Table 42. Trial FB9702-01: Numbers of subjects with biopsies received and suitable for histological scoring

Group (<i>n</i> =3/group)	Skin				Liver				Kidney				Heart			
	LM*		TEM**		LM		TEM		LM		TEM		LM		TEM	
	Pre ¹	Post ²	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0.3 mg/kg every 14 d	2	2	3	3	3	3	3	3	0	0	0	0	0	0	0	0
1.0 mg/kg every 14 d	3	1	3	3	3	3	3	3	0	0	0	0	0	0	0	0
3.0 mg/kg every 14 d	3	3	3	3	3	3	3	3	3	2	3	2	3	3	3	3
1.0 mg/kg every 48 h	1	0	0	0	3	3	0	0	2	2	0	0	3	3	0	0
3.0 mg/kg every 48 h	0	2	0	0	3	2	0	0	2	1	0	0	2	1	0	0

*LM is light microscopy; **TEM is transmission electron microscopy

¹ pre treatment; ² post treatment

Table 43. Trial FB9702-01: Skin superficial capillary endothelial glycolipid scores

Dose group	Subject	Pre-treatment score	Post-treatment score
0.3/14-day	2	3	0
	3	2	0
1.0/14-day	5	3	0
3.0/14-day	7	2	0
	8	2	0
	9	3	0
1.0/48-hour	12	2	0

Table 44. Trial FB9702-01: Heart vascular endothelial glycolipid scores

Dose group	Subject	Pre-treatment score	Post-treatment score
3.0/14-day	7	3	1
	8	3	2
	9	1	0
1.0/48-hour	10	2	0
	11	2	1
	12	1	0
3.0/48-hour	13	1	0

Table 45. FB9702-01: Summary of effect on renal cell types (not including interstitial capillary endothelium)

Cell type	Subjects ID	Effect on light microscopy score
podocyte	8,9	no effect
mesangial cell	8,9	both fell
glomerular endothelial cell	8,9	both fell
arteriolar endothelial cell	9,10,11,13	all fell
arterial medial cell	10,11,13	no effect
arterial intimal cell	10,11,13	no effect (2 were 0 at baseline)
proximal tubular cell	8,9,10,11,13	no effect (all 0 at baseline)
distal tubular cell	8,9,10,13	2 reductions, 2 no effect
collecting duct cell	8,9,10,13	3 reductions, 1 no reduction

Table 46. Trial FB9702-01: Skin and Liver GL-3 levels (ng/mg) and percents reduction

Group/ Subject	Skin			Liver		
	Baseline	Infusion 5	% reduction	Baseline	Infusion 5	% reduction
0.3/ 14-Day						
1	224	262	-17	-	147	-
2	128	83	35	870	74	91
3	198	0	100	1126	48	96
1.0/14-Day						
4	352	42	88	832	185	78
5	262	182	31	176	134	24
6	451	38	92	2406	45	98
3.0/14-Day						
7	454	320	30	371	26	93
8	480	448	7	1776	153	91
9	803	96	88	352	29	92
1.0/48-Hour						
10	332	480	-45	12646	5939	53
11	294	326	-11	1280	38	97
12	422	102	76	1638	77	95
3.0/48-Hour						
13	310	265	15	1690	204	88
14	396	-	-	-	-	-
15	137	25	82	141	0	100

Table 47. Trial FB9702-01: Kidney and heart GL-3 levels (ng/mg) and percents reduction

Group/ subject	Kidney			Heart		
	Baseline	Infusion 5	% reduction	Baseline	Infusion 5	% reduction
3.0/14-Day						
7	3456	-	-	17600	15680	11
8	12928	384	97	38080	40880	-7
9	6912	512	93	17520	12880	26
1.0/48-Hour						
10	1056	448	58	32480	29840	8
11	10144	1984	80	17840	14800	17
12	-	-	-	8320	10720	-29
3.0/48-Hour						
13	1856	5632	-203	24960	48080	-93
14	-	-	-	32960	-	-
15	2336	-	-	-	-	-

Table 48. Trial FB9702-01: Infusion reactions

Subject Number	5	7	8	9
Dose Arm	1 mg/kg q14 day	3 mg/kg q14 day	3 mg/kg q14 day	3 mg/kg q14 day
Infusion Number	4*	5**	4	4
Pretreatment	None	None	None	Ibuprofen
Symptoms	Abdominal discomfort, Nausea, Vomiting, Diaphoretic, Urticaria, Edema Pruritus, Low heart rate	Flushed face, Palpitations, Tachycardia	Shaking chills (responded to diphenhydramine) Febrile when infusion complete	Chills
Treatment	Infusion stopped, Diphenhydramine, Epinephrine, Hydrocortisone	Infusion stopped, Diphenhydramine, Epinephrine, Hydrocortisone	Infusion slowed, Diphenhydramine, Acetaminophen	Infusion slowed, Hydrocortisone
Outcome	Recovered	Recovered	Recovered	Recovered
IgG antibody***	(+)	(+)	(+)	(+)
IgE antibody	(-)	(-)	(-)	Not tested
Skin Tested	(-)	(-)	Not tested	Not tested
Infusion Number	5*	Rechallenge	5*	5*
Pretreatment	Hydroxyzine	Prednisone, Acetaminophen, Diphenhydramine, Methylprednisolone	Acetaminophen	None
Symptoms	Light headed, Stomach ache, Low heart rate	Flushed, Discomfort in throat, Palpitations, Tachycardia	Flushed, Feeling warmth in face, Febrile 20 min. after infusion, Itchy eyes after indomethacin	None
Treatment	Infusion stopped, Epinephrine	Infusion stopped	Indomethacin, Diphenhydramine, Hydrocortisone	N/A
Outcome	Recovered	Recovered	Recovered	N/A

* infusion reactions reported as serious adverse event

** infusion 5 was the last planned infusion in any subject

*** see section in review on antibody development in the trial

Appendix B: Study AGAL-1-002-98**Table 49. Trial AGAL-1-002-98: Enrollment by site**

Site Number	Center, City, and Country	Number of Subjects Enrolled
1	Mount Sinai School of Medicine, NYC, USA	20
2	Beth Israel-Deaconess Medical Center, Boston, USA	3
3	Cedars-Sinai Medical Center, Los Angeles, USA	7
4	Academisch Medisch Centrum, Amsterdam, The Netherlands	2
5	The Middlesex Hospital, London, UK	6
6	Hope Hospital, Manchester, UK	5
7	Hôpital Edouard Herriot, Lyon, France	9
8	Hôpital Broussais, Paris, France	6

Table 50. Trial AGAL-1-002-98: Distribution of baseline scores among those who ended with “0” score*

Baseline score	r-h α Gal	Placebo
3	5	0
2	11	0
1	3	0
0	1	0
Total	20	0

*as-treated population, considered equivalent to intent-to-treat

Table 51. Trial AGAL-1-002-98: Numbers of subjects with “0” or “non 0” score at the end of treatment, by trial center*

	Treatment Group [n, (%)]				Odds ratio	95% C.I.	p-value
	Placebo		r-hαGal				
Site	Zero	Non-zero	Zero	Non-zero			
1	0	10 (100)	8 (80)	2 (20)	0.058	(0.01, 0.24)	<0.001
2	0	1 (100)	2 (100)	0			
3	0	3 (100)	1 (25)	3 (75)			
4	0	1 (100)	1 (100)	0			
5	0	4 (100)	0	2 (100)			
6	0	2 (100)	3 (100)	0			
7	0	5 (100)	3 (75)	1 (25)			
8	0	3 (100)	2 (67)	1 (33)			
Total	0(0)	29 (100)	20 (69)	9(31)			

*as-treated population, considered equivalent to intent-to-treat

The p-value is based on the Mantel-Haenszel χ^2 test stratified by trial center.**Table 52. AGAL-1-002-98: Independent pathologist's judgments of kidney scores on a subset of slides**

Genzyme slide score	Genzyme total	Slide score using Dr. Jennette's scores*			
		0	1	2	3
0	9	8	1	-	-
1	15	-	10	5	-
2	5	-	-	4	1
3	6	-	-	-	6

*Dr. Jennette scored each slide on each of 3 days. The score in the table represents a majority of scores where two scores were different. No slide received scores that differed by more than 1 point on different days.

Table 53. Trial AGAL-1-002-98: Baseline skin and heart biopsy slide scores*

Organ	Slide score	Placebo	r-h α Gal
Heart	0	5	5
	1	21	23
	2	3	1
	3	-	-
	total	29	29
Skin	0	2	2
	1	-	-
	2	15	19
	3	12	8
	total	29	29

*as-treated population, considered equivalent to intent-to-treat

Table 54. Trial AGAL-1-002-98: Tissue GL-3 levels (ng/mg) at baseline and end of trial*

		Placebo			r-h α Gal		
		Baseline	Visit 11	Median % change	Baseline	Visit 11	Median % change
Heart	<i>n</i>	28	28		26	26	
	median	8238	8012	-8	10140	6705	-5
Skin	mean \pm std. error	10796 \pm 1815	9949 \pm 1634		11024 \pm 1740	8850 \pm 1554	
Heart	<i>n</i>	29	28		26	26	
	median	385	343	-24	362	189	-20
Skin	mean \pm std. error	453 \pm 65	414 \pm 62		413 \pm 56	289 \pm 46	

*as-treated population, considered equivalent to intent-to-treat

Table 55. Trial AGAL-1-002-98: Visit at which anti- α galactosidase IgG was first observed (active, as-treated group*)

Treatment	Visit												Total
	0	1	2	3	4	5	6	7	8	9	10	11	
r-h α GAL (n=29)	0	0	0	6	9	2	0	4	0	1	1	1	24
Placebo (n=29)*	0	0	0	0	0	1	0	0	0	0	0	0	1

*Subject 503, who received his first 3 infusions as active treatment and is included in the placebo group, did not develop antibody to α -Gal A.**Table 56. Trial AGAL-1-002-98: Change from baseline as a function of visit number at which IgG seroconversion was noted (r-h α Gal treatment, as-treated group)***

Change score	Visit at which anti- α -Gal A IgG first noted							Total with antibody	Never antibody
	3	4	5	7	9	10	11/13		
-3	0	3	0	0	0	1	0	4	1
-2	6	3	1	3	0	0	0	13	0
-1	0	2	1	0	0	0	1	4	2
0	0	0	0	1	1	0	0	2	2

*omits subject 307, with an imputed worst case score of 3, and an initial score of 1

Table 57. Trial AGAL-1-002-98: Change from baseline as a function of the peak titer (r-h α Gal treatment, as-treated group)*

Change score	Peak IgG titer							
	200	800	3200	6400	12800	25600	51200	102400
-3	0	0	0	0	3	2	1	0
-2	1	1	0	0	2	7	2	1
-1	1	0	0	1	0	0	0	0
0	0	0	1	0	0	0	0	0

*omits subject 307, with an imputed final score

Note that this analysis is tentative, since Genzyme's method for titrating antibody was not qualified.

Table 58. Trial AGAL-1-002-98: Subjects with adverse events, among those adverse events that occurred in at least 2 more subjects in the active group than in placebo, with distributions of severities

WHOART Preferred Term	Placebo n=29				r-h α Gal n=29			
	mild	moderate	severe	total	mild	moderate	severe	total
Post-Operative Pain	10	5	1	16	18	3	1	22
Rigors	4	0	0	4	9	4	2	15
Fever	4	1	0	5	8	6	0	14
Headache	9	1	1	11	8	5	0	13
Rhinitis	6	1	0	7	9	2	0	11
Hematuria	3	3	1	7	10	0	0	10
Anxiety	5	0	0	5	7	1	0	8
Nausea	3	0	1	4	7	1	0	8
Pharyngitis	2	0	0	2	7	1	0	8
Fabry Pain	0	3	0	3	3	1	2	6
Chest Pain	0	2	1	3	2	3	0	5
Edema Dependent	0	1	0	1	4	2	0	6
Pain	2	1	0	3	5	1	0	6
Skeletal Pain	0	0	0	0	4	1	1	6
Temperature Change Sens.*	1	0	0	1	4	0	1	5
Pallor	0	1	0	1	4	0	0	4
Dizziness	2	0	0	2	4	0	0	4
Paresthesia	0	1	1	2	3	0	1	4
Cardiomegaly	1	0	0	1	3	0	0	3
Dyspepsia	1	0	0	1	3	0	0	3
Depression	1	0	0	1	1	2	0	3
Bronchitis	1	0	0	1	1	2	0	3
Eye Abnormality	1	0	0	1	3	0	0	3
Arthrosis	0	0	0	0	2	0	1	3
Hypertension	0	0	0	0	0	3	0	3

*Temperature change sensation refers to feeling warm or cold.

If a subject experienced an adverse event more than once, only the most severe event is tabulated. Remaining events, not listed, were not notably more severe in active than in placebo.

Table 59. Trial AGAL-1-002-98: Infusion-related adverse events (r-h α Gal-treated subjects)

Adverse Events	Subjects with any infusion-related event <i>n</i> =16
Febrile reactions: fever or chills	14
Pain symptoms: Fabry pain or myalgia	5
Hypersensitivity: dyspnea, throat tightness, flushing, chest tightness, pruritis, urticaria, or rhinitis	3
Gastrointestinal: abdominal pain, nausea, or vomiting	3
Cardiovascular: Tachycardia, palpitations, or hypertension	3
Headache	3

Table 60. Trial AGAL-1-002-98: Clinical features of infusion-related events reported to Genzyme Pharmacovigilance as warranting complement or IgE testing*

Subject	Visit #	Additional pre-Treatment ¹	Symptoms	Intensity	Treatment	Infusion rate adjustment (y/n) ²	Time to symptom resolution (hr)
101	4		Shaking chills, hypertension, fever, burning pain	Mod.	Steroids, hydroxyzine, ibuprofen	Y	Shaking/chills 0.75; fever & burning 2.5
	5	ibuprofen	Chills, fever	Mod.	Codeine, ibuprofen	Y	1.5
	6	prednisone	Rigors, fever	Mod.	Unknown	Y	Not provided
	11	prednisone	Shaking chills, hypertension, fever, burning pain	Mod.	Codeine, ibuprofen	Y	Not provided
107	6	-	Shaking chills, fever, pain, upset stomach	Sev.	Ibuprofen, codeine, antacids	Y	1.25
108	5	-	Chills	Mild	Unknown	Y	Not provided
	7	-	Chills, extremity pain	Mild	Ibuprofen	Y	1
112	7	-	Chills, emesis	Mild	Ibuprofen	Y	Chills 0.4
	8	-	Headache, chills, extremity pain, nausea	Mod.	Ibuprofen, codeine, prochlorperazine	Y	Not provided
115	6	-	Shaking chills, extremity pain, fever	Mod.	Ibuprofen, codeine,	Y	Chills 0.5; pain 2
	9	prednisone	Chills, extremity pain	Mod.	Ibuprofen, codeine,	Y	Not provided
	11	prednisone	Shaking chills, extremity pain, fever	Mod.	Ibuprofen, codeine,	Y	0.5-0.75
120	6,7,8,9	-	Chest tightness and shortness of breath	Mild-mod.	none	N	0.25-0.5
	10	-	Chest tightness, shortness of breath, and throat tightness	Mild-mod.	3 liter O ₂	Y	5 min.
	11	-	Shortness of breath	mild	Albuterol inhaler	N	8 min.
202	5,6	-	Itchy, feeling warm	mild	none	N	2
	7	-	Itchy, feeling warm, headaches, hot/cold flashes	unk	Benadryl	N	Itch during infusion spont resolved.
	8	-	Itching, headaches	unk	none	N	Not provided
302	5	-	Chills, muscle tightness, flushed face, hypertension	mild	None	N	0.5: bp in 2
	7	-	Chills, hypertension, fever	unk	none	Y	Not provided
501	8	-	Shivering, cold feeling, hypertension, fever	Mod. or mild	chlorpheniramine	Y	~2
	9	-	Shivering, cold feeling, hypertension, fever	mild	chlorpheniramine	Y	0.5
	10	Chlorpheniramine	Shiver, shaking, feeling cold, wheezing	Mod.	Chlorpheniramine, salbutamol, hydrocortisone IV	Y	1
604	7	-	Shaking, cold feeling	mild	Chlorpheniramine, paracetamol	Y	0.25
	8	-	Sore throat, rigors	mild	chlorpheniramine	Y	Not provided
	9	-	Rigors	mild	chlorpheniramine	Y	0.25
706	10	-	Muscle aches, chills	mild	None	N	0.75
	11	-	Muscle aches, chills	mild	None	N	1
806	9	-	Nausea, vomiting, abdominal pain, decrease in appetite, chills, fever, hypotension, pulse rate increased, paleness, malaise	mild	Zyrtec, polaramine, hydrocortisone IV	Y	>4

*symptoms reported in subjects not reported to Pharmacovigilance: chills (subjects 301 and 702), headache (subject 307), and abdominal pain (subject 804).

¹acetaminophen (paracetamol) and hydroxyzine given for all infusions

²includes interruptions and restarting

*ibuprofen substituted for acetaminophen

Table 61. Trial AGAL-1-002-98: Anti α -Gal IgE and IgG and complement activation in association with the infusion reactions

Subject	Infusion number	IgE test result	IgG test result	Complement activation*
101	4	-	+	"compromised sample"
	5	Not Tested	+	Not Tested
	6	Not Tested	+	Not Tested
	11	-	+	+
107	6	-	+	+
108	5	Not Tested	+	Not Tested
	7	-	+	+
112	7	Not Tested	+	Not Tested
	8	-	+	+
115	6	-	+	+
	9	Not Tested	+	Not Tested
	11	Not Tested	+	Not Tested
120	6,7,8,9	-	+	-
	10	Not Tested	+	Not Tested
	11	Not Tested	+	Not Tested
202	5,6	Not Tested	-	Not Tested
	7	-	-	-
	8	-	-	-
302	5	-	+	+
	7	Not Tested	+	Not Tested
501	8	-	+	+
	9	Inconclusive**	+	+
	10	-	+	+
604	7	-	+	+
	8	Inconclusive**	+	+
	9	-	+	-
706	10	-	+	+
	11	-	+	+
806	9	-	+	+

*tested on serum drawn immediately after or during an infusion reaction

**plasma instead of serum

Appendix C: Study AGAL-005-99**Table 62. AGAL-005-99: McGill short form 18-month results**

Pain Measure	Treatment Group	Entry	18 months Post Entry	Change Entry to 18 months
Sensory (range: 0-33)	Placebo/r-h α Gal	1.7	0.9	-0.8
	r-h α Gal / r-h α Gal	3.0	3.2	0.1
Affective (range: 0-12)	Placebo/ r-h α Gal	0.3	0.2	-0.1
	r-h α Gal / r-h α Gal	0.6	0.8	0.2
Total (range: 0-45)	Placebo/ r-h α Gal	1.9	1.1	-0.9
	r-h α Gal / r-h α Gal	3.6	4.0	0.3
Present Pain Intensity (range: 0-6)	Placebo/ r-h α Gal	0.6	0.4	-0.2
	r-h α Gal / r-h α Gal	0.8	0.5	-0.3
Visual Analog Scale (range: 0-10)	Placebo/ r-h α Gal	0.8	0.4	-0.4
	r-h α Gal r-h α Gal	1.4	1.1	-0.2

Table 63. AGAL-005-99: Change in urinary GL-3 levels (nmol/filter) at 6 months of AGAL-005-99

Statistic	Placebo/r-h α Gal <i>n</i> =22		r-h α Gal/r-h α Gal <i>n</i> =23	
	Entry	% change	Entry	% change
Mean	5357	-43	4091	28
Median	3539	-56	3323	-27
Std. deviation	3812	64	3190	254
Min., Max.	96,12780	-94,209	38,11079	-96, 1170

Baseline data (baseline in AGAL-1-002-98) for these subjects was not provided.

Table 64. AGAL-005-99: Urinary GL-3

Treatment Group	Statistic	Entry	6-Months Post Entry	% Change: Entry to 6-Months Post Entry
Placebo/r-h α Gal	<i>n</i>	22	22	22
	Mean	5357	2546	-43
	Median	3539	1761	-56
	Std. Dev.	3812	2457	64
	Min/Max	96, 12780	81, 8643	-94, 209
R-h α Gal/r-h α Gal	<i>n</i>	23	23	23
	Mean	4091	3050	28
	Median	3323	1966	-27
	Std. Dev.	3190	3294	254
	Min/Max	38, 11079	5.0, 14353	-96, 1170

Table 65. AGAL-005-99: Anti- α -gal IgG and IgE and complement activation in infusion reactions reported to Genzyme Pharmacovigilance during the first 6 months

Treatment	Subject Number	Infusion Number	IgE Test Result	IgG Test Result	Complement Activation
Placebo/ R-h α Gal	0105	9	-	+	-
	0110	7	-	+	+
		13	Not tested	+	Not tested
	0116	4	-	+	+
	0119	8	-	+	Not tested
		11	Not tested	+	Not tested
		12	Not tested	+	Not tested
	0201	3	-	+	+
		4	Not tested	+	Not tested
		5	Not tested	+	Not tested
		6	Not tested	+	Not tested
	0304*	8	-	+	Pre = - Post = +
	0306	5	-	+	+
		7	Not tested	+	Not tested
	0502	8	Not tested	+	Not tested
	0503	10	-	+	+
	0505	10	-	+	+
	0506	13	Not tested	+	Not tested
	0603	5	Not tested	+	Not tested
		10	Not tested	+	Not tested
	0709	5	-	+	+
		7	-	+	+
	0802	6	Not tested	-	Not tested
		8	Not tested	+	Not tested
		10	Not tested	+	Not tested
		11	Not tested	+	Not tested
	0805*	4	-	+	+
		7	-	+	+
		8	-	+	+
		9	Not tested	+	Not tested
		10	-	+	Pre = + Post = +
R-h α Gal/ R-h α Gal	0104	1	-	+	+
		7	Not tested	+	Not tested
	0107	3	Not tested	+	Not tested
		5	-	+	-
		6	-	+	+
		7	Not tested	+	Not tested
	0115	13	Not tested	+	Not tested
	0202	2	Not tested	-	Not tested
		3	Not tested	-	Not tested
		8	-	-	Not tested
	0402	12	-	+	+
	0501	4	-	+	+
		7	-	+	+
		8	-	+	+
		9	-	+	+
	0604	11	Not tested	+	Not tested
		1	Not tested	+	Not tested
		3	Not tested	+	Not tested
		8	Not tested	+	+
	0706	1	Not tested	ND	Not tested
		7	Not tested	+	Not tested
		9	Not tested	+	Not tested
	0804	3	Not tested	+	Not tested
		5	-	+	+
		6	Not tested	+	Not tested
	0806	1	Not tested	+	Not tested
		10	Not tested	+	Not tested

*Subjects 304 and 805 had skin testing for suspicion of the development of serum IgE. Subject 304 was withdrawn because he developed a (+) skin test; subject 805's test was (-).

Table 66. Physiological testing in AGAL-1-002-98 and AGAL-005-99 (18 months)

Scale	Treatment group	AGAL-1-002-98		AGAL-005-99				
		Baseline	Change to end	entry	Change			
					entry to 6 months	baseline to 6 months	entry to 18 months	baseline to 18 months
TDT Mean just noticeable difference	Placebo/r-h α Gal	19.1	3.2	22.4	1.5	5	1.1	4.3
	r-h α Gal / r-h α Gal	24.1	3.8	27.8	-0.3	0.1	-5.5	-5
TDT Mean Threshold	Placebo/ r-h α Gal	13.7	5.3	19	0.6	5.8	-4.1	0.4
	r-h α Gal / r-h α Gal	19.5	5.1	24.6	-1.6	-0.7	-7.5	-6.5
QSART (Mean Sweat Volume)	Placebo/ r-h α Gal	65	-22.8	42.1	1.3	-22.8	-24.3	-55.4
	r-h α Gal / r-h α Gal	37.8	18.2	55.9	-29.7	-12	-39.3	-25.6
VOP - Mean Baseline Flow	Placebo/ r-h α Gal	5.6	0.5	6.2	-1	-0.9	-0.8	-1.2
	r-h α Gal / r-h α Gal	5.5	1.4	6.8	-2	0	-1.2	0.8
VOP – Mean First Peak	Placebo/ r-h α Gal	14.9	3.3	18.2	0.1	2.3	-4.3	-2.1
	r-h α Gal / r-h α Gal	14	3.4	17.4	-0.3	2.9	-4.5	-2.6
VOP - Mean Second Peak	Placebo/ r-h α Gal	9.2	1.8	11	-0.4	0.9	-4.2	-3.1
	r-h α Gal / r-h α Gal	8.7	2.8	11.5	-3.1	0.1	-4	-0.6
VOP - Mean Third Peak	Placebo/ r-h α Gal	7.1	2	9.1	-0.6	1	-2.8	-1.7
	r-h α Gal / r-h α Gal	7	3.1	10.1	-2.7	0.9	-4.3	-0.5
VOP – Mean Fourth Peak	Placebo/ r-h α Gal	6	1.1	7.2	-0.1	0.7	-1.7	-1.3
	r-h α Gal / r-h α Gal	6	3.1	9.1	-2.9	0.8	-3.5	-0.1

Table 67. AGAL-005-99: Physician assessment of Fabry symptoms, numbers (%)

		entry	18-month score		total
			yes	no	
Angiokeratoma	Placebo/r-h α Gal	Yes	22	2	24
		No	0	1	1
	R-h α Gal /r-h α Gal	Yes	23	0	23
		No	0	2	2
Abnormal Sweating	Placebo/r-h α Gal	Yes	19 (95)	1 (5)	20
		No	2 (40)	3 (60)	5
	r-h α Gal / r-h α Gal	Yes	19 (95)	1 (5)	20
		No	1 (20)	4 (80)	5
Abdominal Pain	Placebo/ r-h α Gal	Yes	3 (75)	1 (25)	4
		No	4 (19)	17 (81)	21
	r-h α Gal /a r-h α Gal	Yes	5 (83)	1 (17)	6
		No	5 (26)	14 (74)	19

Table 68. AGAL-005-99: 18-month adverse events by preferred term, occurring in ≥ 3 subjects overall

Preferred term	Placebo /r-h α Gal (18 months exposure) n=29	R-h α Gal/r-h α Gal (23 months exposure) n=29
Rhinitis	19	22
Rigors	18	16
Albuminuria	15	13
Fever	15	12
Headache	10	17
Coughing	9	15
Renal Function Abnormal	14	9
Pain	13	9
Temperature Changed Sensation*	13	9
Heart Valve Disorders	10	11
Vomiting	7	14
Nausea	6	13
Cardiomegaly	7	11
Pharyngitis	9	9
Upper Resp Tract Infection	9	9
Anemia	8	9
Post-Operative Pain	8	9
Chest Pain	11	5
Edema Dependent	9	7
Abdominal Pain	7	8
Fabry Pain	9	6
Influenza-Like Symptoms	8	7
Paraesthesia	5	9
Back Pain	6	7
Myalgia	6	7
Fatigue	7	5
Heart Disorder	8	4
Asthenia	5	6
Bradycardia	6	5
Bronchitis	4	7
Dyspnea	6	5
Pruritus	6	5
Diarrhea	6	4
Dizziness	4	6
ECG Abnormal	3	7
Somnolence	5	5
Anxiety	6	3
Depression	3	6
Dyspepsia	6	3
Heart Block	4	5
Bronchospasm	4	4
Flushing	7	1
Retinal Disorder	4	4
Skeletal Pain	4	4
Vision Abnormal	3	5
Cardiac Failure	4	3
Hematuria	3	4
Hypertension	3	4
Malaise	4	3
Rash	2	5
Acne	3	3
Bundle Branch Block	3	3
Hearing Decreased	3	3
Infection	5	1
Injury Accident	3	3
Leg Pain	3	3
Palpitation	4	2
Tremor	4	2

*Temperature changed sensation refers to feeling warm or cold.