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Memorandum

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Division of Clinical Trial Design and Analysis
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FROM: James Kaiser, M.D.

THROUGH: Marc Walton, M.D., Ph.D.

SUBJECT: Medical Officer's Review
BLA STN 103979/0
Applicant: Genzyme
Product: recombinant human α -Galactosidase A
Proposed indication: long-term enzyme replacement

ATTACHMENT: memo from Dr. J. Charles Jennette, M.D., with an evaluation of kidney, skin, and heart biopsy slides from Genzyme clinical trial AGAL-1-002-98

TO: BLA STN 103979 /0 file

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SYNOPSIS OF APPLICATION

Genzyme submits this application for marketing approval for their recombinant human α -Galactosidase (r-h α Gal) in the treatment of Fabry's disease. Alpha-Galactosidase A has been granted orphan drug and fast-track designation. Genzyme has requested that their product be considered under the accelerated approval mechanism.

BACKGROUND

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. 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 in the past was 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, respectively³). The deficiency of α -gal activity may cause an increase in plasma substrate levels; a published estimate of the normal level of GL-3 is 0.9 ± 0.4 μ g/ml.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. 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 r-h α Gal is produced in Chinese hamster ovary (CHO) cells transfected with a vector carrying the cloned cDNA of the human α -Galactosidase gene. It is a disulfide-linked homodimer with an approximate molecular weight of 100 kilodaltons. Each of the approximately 51 kDa subunits contains three N-linked glycosylation sequences. Specific activity is between --- and -- - units/mg, where a unit is defined as that amount of activity that results in hydrolysis of 1 micromole of a synthetic substrate, p-nitrophenyl-I-D-Galactopyranoside, per minute under specific assay conditions.

For the pre-pivotal trial (FB9702-01) and for preclinical acute toxicity studies, r-h α Gal was manufactured at 30 liter and 160 liter bioreactor scales. For the 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. The judgment of the product reviewer is that the Drug Substance at each scale is biochemically comparable.

The product is formulated as a lyophilized powder that when reconstituted with water for injection yields a clear solution containing r-h α GAL 5.0 mg/ml, mannitol 30.0 mg/ml, sodium phosphate monobasic monohydrate 2.75 mg/ml, and sodium phosphate dibasic heptahydrate 8.0 mg/ml. It further requires dilution with normal saline "or equivalent" solution for slow intravenous administration.

The name given to the product by the United States Adopted Names council (USAN) is agalsidase beta.

CLINICAL TRIALS CONDUCTED AND PLANNED, AND SCOPE OF REVIEW

Genzyme has completed two clinical trials of r-h α Gal, both in subjects with Fabry's disease. The first, FB9702-01, was an open-label, 15-subject trial studying 5 different dose regimens. The second trial, AGAL-1-002-98, was a placebo-controlled, randomized, 5-month, double-blind trial in 58 subjects examining a surrogate endpoint, levels of substrate in renal vasculature as measured histologically). A third trial, AGAL-005-99, an open-label extension to AGAL-1-002-98, is ongoing, and contains important evidence on the durability of the effects seen in the controlled trial. FB9702-01, AGAL-1-002-98, and 6-month results from AGAL-005-99 are reviewed in this document. In addition, Genzyme provides serious adverse event data from approximately 30 additional subjects at the proposed dose in various trials, including AGAL-006-99, the open-label extension to FB9702-01 (see 120-day safety update).

As AGAL-1-002-98's primary endpoint is a surrogate Genzyme has requested consideration of the application under accelerated approval and is in the process of conducting an additional controlled trial with clinical endpoints, to study whether their α -galactosidase product confers clinical benefit.

The current submission contains product, preclinical, and human data. The human data include pharmacology and safety and efficacy information. This document is a review of the human safety and efficacy data.

TRIAL: FB9702-01

Pharmacokinetic and pharmacodynamic evaluation of recombinant human α -Galactosidase A (r-h α Gal) replacement in patients with Fabry disease

DESIGN

This was a single center (Mt. Sinai Medical Center, New York City), 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.

Subject qualifications

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

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 for a total of five doses; two groups of three patients each were to receive r-h α Gal at 1.0 and 3.0 mg/kg every 48 hours for a total of five doses. 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 supplied as a lyophilate containing 15 mg of enzyme in a 5 ml vial, to be reconstituted with 3.2 ml of sterile water for injection and further diluted for slow intravenous infusion. The infusion was to be given at 0.83 ml/minute (over 2 hours). The every-14-day cohorts were to be enrolled sequentially, starting with the lowest dose, then the every-48-hour cohorts were to be enrolled sequentially, starting with the lower dose. There was no limitation on concomitant medications. The final evaluation was to be 2 weeks after the last dose.

Product used for the first infusion in Groups ----- was produced at the 30 liter scale. Material used for the first infusion for all but one patient (Patient ---) in Group -- and all patients in Group -- was produced at the 160 L scale.

Procedures and evaluations

Important assessments and procedures were:

- Pre-study, to be completed within 30 days of r-h α Gal infusion:

- Medical history, demographics, and physical examination including vital signs
- 12-lead ECG
- plasma GL-3 and α -gal
- IgG antibody to r-h α Gal
- Dispense daily diary
- Concomitant medication assessment
- Baseline assessments, to be completed within 6 days prior to 1st infusion
 - plasma GL-3 and α -gal
 - liver and skin biopsy
 - optional kidney, small intestine, and endomyocardial biopsy
 - 24-hour urine collection for creatinine clearance and GL-3
 - echocardiogram, signal averaged ECG
 - cardiac and abdominal MRI
 - ophthalmic examination
 - quality of life questionnaires
 - thermal discrimination tests
 - sympathetic skin responses and pilocarpine-induced sweating tests for subjects on 48-hour treatment schedule
 - examine daily pain questionnaire
 - adverse events and concomitant medications
- Day 0 (1st infusion)
 - hematology and serum chemistries at 2 and 24 hours after infusion
 - plasma GL-3 and α -gal
 - infusion with measurement of vital signs and r-h α Gal levels
 - examine daily pain questionnaire up to 48 hours after infusion 1
 - record adverse events and concomitant medications (up to 48 hours after infusion 1)
- 2-3 days after infusion 1:
 - subjects on 14-day infusion schedule only: optional liver and skin biopsy
- Infusions 2-4 (days 14, 28, and 42 for subjects on the every-14-day schedule, and days 2,4, and 6 for subjects on the every-48-hour schedule)

The following were to be performed during the 24 hours after infusion:

 - vital signs
 - hematology and serum chemistries
 - ECG
 - plasma GL-3 and α -gal
 - examine daily pain questionnaire
 - adverse events and concomitant medications
- Final infusion day (day 56 for subjects on the every-14-day schedule, and day 8 for subjects on the every 48-hour schedule)
 - ECG
 - hematology and serum chemistries at 2 and 24 hours after infusion
 - plasma GL-3 and α -gal
 - infusion with measurement of vital signs and r-h α Gal levels
 - echocardiogram, signal averaged ECG
 - cardiac and abdominal MRI
 - ophthalmic examination
 - quality of life questionnaires
 - thermal discrimination tests

- sympathetic skin responses and pilocarpine-induced sweating tests for subjects on 48-hour treatment schedule
- examine daily pain questionnaire
- adverse events and concomitant medications
- 2-3 days after infusion 5:
 - liver and skin biopsy
 - 24-hour urine collection for creatinine clearance and GL-3
- 2-4 days after infusion 5
 - optional kidney, small intestine, and endomyocardial biopsy
- 14 days after infusion 5:
 - subjects on 48-hour infusion schedule only: optional liver and skin biopsy
 - plasma GL-3 and α -gal
- Trial completion (21-28 days after final infusion)
 - physical examination
 - plasma GL-3 and α -gal
 - hematology and serum chemistries
 - IgG antibody to r-h α Gal
 - ECG
 - Adverse events and concomitant medications

In summary, 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.

The optional kidney and endomyocardial biopsies were to be done for selected subjects (48-hour treatment schedule and every 14-day, 3.0 mg/kg subjects). Optional small intestinal biopsies were only to be for those with gastrointestinal symptoms.

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 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. Histology slides were read by a Mt. Sinai pathologist expert in the organ in question.

Total GL-3 content was to be determined at Genzyme.

Analysis

The protocol specified the following as outcomes:

- Safety
- Pharmacokinetics
- GL-3 levels in plasma and skin.
- Clinical outcomes as questionnaires and other parameters

The final analysis of the trial was to be descriptive.

Comments

Pharmacokinetics are not reviewed in this document.

Genzyme reported pharmacodynamic results for skin, liver, heart, and kidney tissue as levels of GL-3 determined biochemically and histologically. These are reviewed here.

FB9702's open-label design rendered it inadequate to give meaningful data on clinical effects of r-haGal. 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-haGal prior to their final evaluations than those every-14-day infusion groups. Thus the trial was not designed to give direct information on the durability of the response to r-haGal given the more frequent dosing regimen.

RESULTS: CONDUCT OF TRIAL

The trial protocol was made final on March 5, 1998, and the first subject was enrolled on April 21, 1998. It was amended once after it was implemented, in an amendment dated August 11, 1998, which added the optional duodenal/jejunal biopsy, added clinical measures (signal-averaged ECG, ophthalmic examination), and added hypersensitivity management procedures.

In addition, Genzyme implemented the following changes to the conduct of the trial:

- While samples for plasma GL-3 and α -Gal A were obtained at the beginning of the study, historical values were used to determine patient eligibility.
- Echocardiograms were not performed at study completion.
- 24-hour urine collections were increased to 48 hours at the discretion of the investigator.

Genzyme did not present the timing of the implementation of these changes.

Comment

The changes to the trial outlined above would not be expected to change the assessment of the results of the trial substantially.

Eligibility, dosing, and other protocol violations

Eligibility violations were rare. Plasma GL-3 levels in 2 subjects were below those specified as entry criteria (<5.0 ng/ μ l), due to historical data being used for entry criteria.

Seven subjects had liver biopsies taken outside the 2-3 day time window after the last infusion (variable time periods); 1 subject had no liver biopsy. Six subjects had skin biopsies taken outside the 2-3 day time window after the last infusion (variable time periods); 1 subject had no skin biopsy.

There were numerous deviations from exact schedules of collection of data and incomplete collection of data, including MRIs and ECGs, ophthalmology examinations, urine collections, sweat tests and thermal discrimination tests, and questionnaires. Pharmacokinetic sampling prior to the first infusion failed to fall within a prespecified time window in all but 1 subject. The date of the final evaluation fell outside the 21-28-day time window for numerous subjects.

With a few exceptions, dosing was complete. Subject 5 in the 1 mg/kg, every-14-day infusion group received partial doses at infusions 4 and 5, and subject 7 in the 3 mg/kg, every-14-day infusion group received a partial dose at infusion 5. The infusion for subject 7 was attempted again 2-weeks later but it, too, could not be completed. These infusions were partial because of the occurrence of infusion reactions (see safety discussion below).

Comments

The determination of safety from the trial would not have been significantly compromised by the protocol deviations summarized above. I will defer discussion of the impact of the inexact timing of blood draws on the pharmacokinetic and pharmacodynamic analysis to the PK/PD reviewer. The impact of the inexact timing of biopsies is hard to gauge, since the kinetics of the reduction or

reaccumulation of substrate are not known. However, there was no impact of differences of timing of final biopsies in AGAL-1-002-98 (see sensitivity analyses to the primary endpoint data).

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.

Subject disposition

The trial enrolled the number of subjects planned, and all 15 subjects completed the trial.

RESULTS: DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Table 1 shows pertinent demographics and baseline characteristic data. The trial population was entirely males and mostly "White." The two subjects below the inclusionary limit for plasma GL-3 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.

Table 1. Trial FB9702-01: Demographics and baseline characteristics

	0.3/14-day n=3	1.0/14-day n=3	3.0/14-day n=3	1.0/48-hr n=3	3.0/48-hr n=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 (n) male	3	3	3	3	3
Race (n)					
White	3	1	2	2	3
Black	0	0	0	0	0
Hispanic	0	2	1	1	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

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: EFFICACY

Table 2 shows Genzyme's summary of the number of subjects (out of 3 per cohort) with samples suitable for histological analysis for each organ.

Table 2. Trial FB9702-01: Numbers of subjects with biopsies received and suitable for histological scoring

Group (n=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

Comment

Biopsy data were incomplete in most cases except for liver light microscopy, rendering comparisons from the every 14-day to every 48-hour groups tenuous for most organs. The number of subjects for whom data are reported does not correlate with the above table in every instance (for example, see results for skin below).

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 in the BLA, appear to show reductions.

Review of scores of transmission electron micrographs of liver shows that sinusoidal endothelial and Kupffer GL-3 was reduced in nearly all subjects (13/14 for whom both baseline and end of treatment biopsy results were available for both cell types), with reductions in hepatocytes in 7/14 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.

Skin histology

The extent of skin biopsy light microscopy scoring was variable: while all 15 patients had pre- and post-treatment skin samples, not every specimen received a coded score due to low vessel numbers or uncertain classification on the part of the pathologist. Table 3 shows light microscopy scores for the superficial capillary endothelium of the skin, where pre- and post-treatment scores were available.

Table 3. 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

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

Although listings contain paired evaluations for the subjects reported above, the summary table of numbers of samples adequate for analysis does not show this number of adequate samples. The cause for this discrepancy is not clear.

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 biopsies for which baseline and end-of-treatment samples were available (Table 4).

Table 4. 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

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

Kidney slides were scored separately for various cell types. Table 5 shows data for interstitial capillaries in subjects with both baseline and end-of-treatment scores. Four of the 5 subjects had a reduction in score.

Table 5. 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)

The following is a summary of the light microscopy results for other cell types in the kidney.

- Only 2 subjects, numbers 8 and 9, had samples in which podocytes, glomerular mesangial cells, and glomerular endothelial cells were scored at baseline and end of treatment.
 - There was no effect of treatment on podocytes, which were scored as 3 for both subjects
 - mesangial cell lipid accumulation fell from 2 to 0 in both cases
 - glomerular endothelial cell scores fell from 2 and 1 to 0.
- Subjects 9, 10, 11, and 13 had samples in which arteriolar endothelial cell GL-3 was scored at baseline and end of treatment: the scores fell for these subjects, from 3 to 1 in one subject and from 2 to 1 in three subjects, respectively.
- Subjects 10, 11, and 13, had samples in which arterial medial cells were scored at baseline and end of treatment: the scores for these didn't change (1 and 2 (twice)).
- Subjects 10, 11, and 13, had samples in which arterial intimal cells were scored at baseline and end of treatment: 2 were 0 at baseline and 1 was scored 1 at baseline; none changed.
- Subjects 8, 9, 10, 11, and 13 provided pairs of proximal tubular cell scores: all were 0 at baseline and stayed 0.
- Subjects 8, 9, 10, and 13 provided pairs of distal tubular cell scores: there were 2 reductions and 2 pairs that showed no reduction (none were 0 at baseline).
- Subjects 8, 9, 10, and 13 provided pairs of collecting duct cell scores: there were 3 reductions and 1 pair that showed no reduction (none were 0 at baseline).

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 on histology in various organs

Available data showed a reduction in lipid in endothelium in the kidney, heart, and skin, but data in the liver were sparse. 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. Table 6 shows data for skin and liver, the organs examined for all dose cohorts. 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.

Table 6. 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 7 shows GL-3 data for the kidney and heart, which were not studied in the two lowest every 14-day groups. The data are too sparse to draw conclusions about dose level or frequency. Reduction occurred in most renal tissues examined; effects on the heart were quite variable.

Table 7. 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	-	-	-	-	-

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 (Table 8). 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. The 3mg/kg, every-48-hour infusion group had a very low baseline GL-3 level (2 subjects in the cohort were enrolled with levels below the inclusion criterion) and did not contribute to the understanding of dose and clearance.

Table 8. Trial FB9702-01: Plasma GL-3 levels (ng/ml)

Dose Group <i>n=3 each</i>	Pre-infusion 1 Mean (range)	Pre-infusion 2* Mean (range)	Pre-infusion 5** Mean (range)
0.3/14 day	18.2 (15.8 - 20.4)	11.9 (10.8 -13.8)	3.0 (0.9 - 5.0)
1.0/14 day	15.7 (12.5 - 20.3)	1.8 (0.0 – 5.0)	3.0 (0.0 - 8.6)
3.0/14 day	34.1 (20.3 - 53.9)	1.2 (0.6 -1.7)	0.8 (0.0 - 1.6)
1.0/48 hour	13.2 (3.0 – 23.5)	39.9 (16.2 – 80.0)	9.8 (4.6 – 13.0)
3.0/48 hour	4.3 (2.0 – 7.4)	3.8 (0.0 -11.4)	0.0 (0 - 0)

*day 14 for the every-14 day group; day 2 for the every-48 hour group

**day 56 for the every-14 day group; day 8 for the every-48 hour group

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.

Comment

Genzyme does not detail the nature of the inconsistency that renders the quantification of urine GL-3 inexact. Although Genzyme concludes that a trend toward reduction in urinary GL-3 is shown, this conclusion cannot be verified.

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, without 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. Results were not consistent among all subjects within each dose groups, and thus show 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

For a detailed review of the pharmacokinetics substudy, see the review of the clinical pharmacologist. Genzyme measured serum levels of r-h α Gal around the 1st and 5th infusions. The conclusion of the pharmacologist is that there were no meaningful differences in pharmacokinetic measures dependent upon infusion number within each dose and regimen, that exposure increased out of proportion to dose, and that the data suggest that the pharmacokinetics did not change with the development of antibodies to r-h α Gal. Terminal half-life varied between 54 and 94 minutes.

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 (see the section “Eligibility, dosing, and other protocol violations” for a full description). The duration of infusions was about 2 hours in all dosing groups except the 3 mg/every-48 hour group. In this group, the mean was about 5 hours, since each subject had his infusion time extended due to investigator concerns. Table 9 shows total r-h α Gal exposure in the trial.

Table 9. FB97002-01: Total exposure (mg) by dosing group

0.3/q 14 day <i>n</i> =3	1.0/q 14 day <i>n</i> =3	3.0/q 14 day <i>n</i> =3	1.0/q 48 hour <i>n</i> =3	3.0/q 48 hour <i>n</i> =3
21.2 (16.0 - 26.8)	80.3 (27.0 - 99.2)	222.6 (84.8 - 341.3)	70.2 (65.0 - 74.2)	244.2 (199.8 - 304.0)

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 diagnosed by symptoms and a high-probability ventilation/perfusion scan. Anticoagulation administered due to a history of deep venous thrombosis had been discontinued prior to the subject receiving r-h α Gal. A pre-study Doppler examination showed no active thrombotic disease. Two days after receiving the last infusion, he was diagnosed with pulmonary emboli. After anticoagulation therapy, two months later he had a low-probability ventilation/perfusion scan.

Comments on serious adverse events

For a discussion of the serious infusion reaction, see the section on infusion reactions.

Regarding the subject with pulmonary emboli, there are no other data in the safety data base that suggest thrombotic events; the one event in this trial is insufficient evidence for concern, considering that it occurred in a person with a predisposition to the syndrome who had been taken off treatment for it.

Infusion reactions

Infusion reactions were reported in 4 subjects, 3 of whom were in the every-14-day 3 mg/kg dose group. Table 10 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. Skin testing for IgE against r-h α Gal was to be done for suspected hypersensitivity reactions.

Table 10. 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

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 11), so 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. Durations were recorded for 13 subjects; for 7 subjects, the events were from 11-115 days; for the others the events lasted less than a day. The events were mild in 10 subjects and moderate in severity in 4.

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

There was only 1 event coded as severe: the pulmonary embolism reviewed previously. The most common "moderate" adverse events were hypertension (4 subjects), allergic reaction and fever (3 subjects each), and abdominal pain (2 subjects).

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

Medications taken after the initiation of the trial included those for infusion reactions, for biopsy sedation, and post-biopsy pain, and cutaneous antibiotic prophylaxis. Data on concomitant medication use was searched for indications applying to hypertension. With the terms “increased blood pressure” and “hypertension” as search criteria, 1 subject was noted to have started an antihypertensive after the initiation of the trial. 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

Genzyme screened 200 normal persons and developed a criterion normal value for an ELISA detecting serum IgG antibody against r-h α Gal. Table 11 shows that the development of antibodies was common.

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

Table 11. Trial FB9702-01: Development of IgG antibody against r-ha Gal

Dose group (mg/kg)	Subject	ELISA*		Western Blot**	
		Prior to infusion 1	Up to trial completion	Prior to infusion 1	Up to trial completion
0.3/ q2 weeks	1	wnr	wnr	faint +	faint +
	2	wnr	wnr	-	-
	3	wnr	ANR	-	-
1.0/ q2 weeks	4	wnr	ANR	-	+
	5	wnr	ANR	-	+
	6	wnr	ANR	faint +	+
3-0/ q2 weeks	7	wnr	ANR		+
	8	wnr	ANR		+
	9	ANR	ANR	faint +	+
1.0/ q48 hours	10	wnr	ANR	-	+
	11	wnr	wnr	-	-
	12	wnr	wnr	-	-
3-0/ q48 hours	13	wnr	ANR	-	-
	14	wnr	wnr	-	-
	15	wnr	ANR	-	+

*wnr=within normal range; ANR =above normal range (determined on 200 samples from normals)

**expressed as negative (-), positive (+), or faint positive

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.

TRIAL: AGAL-1-002-98

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

DESIGN

AGAL-1-002-98 was a multinational, double-blind, randomized, placebo-controlled, multiple-dose trial to study about 60 individuals with Fabry's disease. Its primary objectives were to determine efficacy in terms of reduction of enzyme substrate from kidney, heart, and skin tissue, and to determine 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 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 supplied as lyophilates to be reconstituted with 7.2 ml of sterile water for injection and further diluted with a 0.9% sodium chloride solution to a final total volume of 500 ml for slow intravenous infusion. 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 to be supplied in identical 20 ml vials; vial labeling was to be blinded prior to sending to trial sites. Treatment assignments were not to be reused in case of the discontinuation of a subject prior to completion of the trial. Randomization codes were not to be accessible to Genzyme personnel directly involved with the trial, but could be available to other Genzyme personnel.

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 a-gal
 - r-haGal antibody
 - GL-3 level and plasma a-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 a time window of 3 days before and after each study visit. Study personnel were to call subjects at 1-week intervals between study visits to ensure completion of the diary on a daily basis.

- Visits 1-6, 8-10
 - Trial agent infusion
 - r-haGal antibody (prior to infusion)
 - visits 4 and 10 only: clinical chemistry, hematology, and urinalysis
 - Pharmacokinetics (at or before visit 3, European patients only)
 - Short Form McGill Pain Questionnaire, Fabry Symptom Assessment, Total Symptom Score
 - Adverse event and concomitant medication monitoring
 - Review and dispensing of patient diary

- Visit 7 (day 84 \pm 3 days)
 - Trial agent infusion
 - r-haGal antibody (prior to infusion)
 - Clinical chemistry, hematology, serum cystatin C, and urinalysis
 - Pharmacokinetics (European patients only)
 - Plasma GL-3 (prior to study drug infusion)
 - Physical examination
 - 12-lead ECG
 - Short Form McGill Pain Questionnaire, SF-36 Health Survey, Fabry Symptom Assessment, Total Symptom Score
 - Adverse event and concomitant medication monitoring
 - Review and dispensing of patient diary

- Visit 11 or final trial visit (day 140)—also applicable to those discontinuing before completion of all infusions
 - 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

There was a 28-day window after the final study visit for the following evaluations, including the biopsy performed for the primary endpoint, unless otherwise specified:

- 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.

Details of the method of selection of the kidney biopsy samples are provided in the BLA. 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 populations for analysis: 1) intent-to-treat (ITT) population, in which subjects are assigned to the treatment to which they were intended to be randomized; 2) "as treated," in which subjects are assigned for analysis to the treatment that they actually received; and 3) "per protocol," defined as the subset of the "as treated" population that actually received at least one infusion, did not have a score of "0" at baseline, and did not have any major protocol violations. The ITT population was the primary population for analysis.

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.

Scoring method for endothelium

Kidney interstitial capillary endothelium was initially evaluated using a qualitative method that had not received concurrence from CBER. Slides received scores of 0, 1, 2, or 3 ("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 (In case of discrepant scoring of 2 or greater, the pathologists would reconvene to reach agreement regarding the score or record new scores. This procedure resulted in an "adjudicated" score).

CBER asked for a quantitative analysis in order to obtain verifiable slide scores. Following discussions, Genzyme submitted a new blinded analysis of slides with a score of 0 or 1 from the

initial reading (post-adjudicated if necessary). The new pathology reading procedure was codified in a document dated March 29, 2000. In this re-reading, all capillaries on the slide were examined. The slide score was based upon an algorithm for the counting of these scores. The following are important aspects of the scoring system:

1. Selection of section to be examined: The first pathologist selected the more technically readable section of the two that were mounted on a slide. If the number of capillaries on the chosen section was less than 50, both sections would be evaluated.
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

Following review of the light microscopy, transmission electron micrographs were to be reviewed, but did not figure into the score for the slide. Discrepancies in appearance were to be noted, however.

3. Slide score: Using an algorithm that included severity scores for each capillary scored on a given slide, a new slide score of 0 or 1 was rendered, where 0 was “nearly clear” 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 normal could have theoretically had 4.9% of its capillaries scored 3.
 - b. 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.”
 - c. The slide would receive the score that the majority of pathologists gave it.

Analytical methods and missing values

The primary analysis of the primary endpoint was pre-specified in the protocol as a χ^2 test of the proportion of subjects with a score of “0” on the light microscope assessment of capillary endothelium. ANOVA was specified as a possible additional analysis.

The protocol specified that missing slide scores would receive a worst-case value of 3. This occurred only for the end-of-treatment slide for active-treated subject 307.

Secondary endpoints

Secondary endpoints were generally grouped into pain measurements and quantitation of GL-3.

- A pain endpoint was really 5 endpoints. It was the McGill short form pain questionnaire change from baseline to visit 11 in
 - 1) sensory pain score
 - 2) affective pain score
 - 3) total pain score
 - 4) visual analog scale score

- 5) present pain intensity
- Another endpoint included primary endpoint results:
 - 6) Quantitation of GL-3 accumulation in the capillary endothelium of the heart, kidney, and skin as determined light microscopy comparing baseline to 20 weeks
- Total GL-3 measured by ELISA was really two endpoints:
 - 7) change from baseline to visit 11 in urine GL-3
 - 8) change from baseline to visit 11 in kidney tissue GL-3

Analytical methods

The protocol stated that the analyses of the secondary endpoints would be similar to that of the primary endpoint. Further details were not provided.

Tertiary endpoints

There were many tertiary endpoints, all measuring a difference from baseline to end of trial:

- 1) Vibration Detection Threshold
- 2) Neuropathy Impairment Score
- 3) Neuropathy Symptoms and Change Score
- 4) Total Symptom Score
- 5) SF-36 "quality of life" questionnaire
- 6) Physician's assessment of Fabry symptoms
- 7) Symptom-free days as assessed by diary
- 8) Episode free days as assessed by diary
- 9) Mean pain score from diary
- 10) Glomerular filtration rate
- 11) Autonomic score based on Quantitative Sudomotor Axon Reflex Test, Thermal Detection Threshold Just Noticeable Difference score, and Venous Occlusion Plethysmography

"Other" endpoints

- 1) Ophthalmic examination
- 2) Urinary protein and creatinine ratios
- 3) Plasma GL-3
- 4) Renal plasma flow

Comments on the protocol

Genzyme's previous results suggested that a brief trial such as this one 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. Other supportive determinations, such as pain measurement and serum creatinine, were performed more frequently, so the kinetics of a possible benefit could have been inferred from these endpoints.

RESULTS: CONDUCT OF TRIAL

Dates of the trial

The trial started on March 14, 1999, and was completed on February 4, 2000.

Protocol changes

The protocol underwent significant revisions, including those to trial duration, endpoints, and endpoint methods of analysis. Five amendments were submitted after the trial was started. The following is a synopsis of the major points in each amendment:

- Amendment dated May 5, 1999, increased enrollment from about 50 to about 60 and added some tertiary endpoint measurements.
- Amendment dated May 14, 1999, added a pharmacokinetic substudy for subjects participating through European sites.
- Amendment dated July 19, 1999, shortened the trial by a month, reducing the number of biweekly infusions from 13 to 11, with final followup at day 140.
- Amendment dated November 12, 1999, changed the endpoints of the trial. Prior to this the endpoint was a composite score combining morphological and biochemical assessments of GL-3 in skin, heart, and kidney combined. With this amendment the primary endpoint was restricted to the quantitation of GL-3 in kidney alone.
- Amendment dated April 14, 2000, shortened and established the final ordered set of secondary endpoints; tertiary endpoints were increased in number. This amendment formalized histological examination and analysis methods, agreed-to with CBER, for the rereading of kidney biopsy slides (Genzyme's proposed histological method for review of heart and skin biopsy slides was not altered). A reading of biopsy slides, for the primary and secondary endpoint, had already been performed according to a method upon which agreement had not been reached. The new methods constituted the agreed-to final data set for the primary analysis.

Comments

The major changes to the endpoint of the trial were the result of extensive discussions between Genzyme and CBER, and were not prompted by a review of the data by Genzyme. The method of assessing the primary endpoint was changed from a subjective scoring technique to one in which individual capillaries were scored, rendering the results much more able to be analyzed. Another major change to the trial was its shortening from 6 to 5 months, which occurred before any subject could have been assessed for a primary endpoint.

Enrollment by site

Table 12 shows the enrollment by site. Fifty-eight subjects were enrolled. The Mount Sinai site had the most subjects by a large margin.

Table 12. 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

Discontinuations

No subject discontinued participation in the trial.

Protocol violations**Treatment assignments**

Reversals of treatment assignment occurred in 6 subjects in the trial, 3 for each treatment group (Table 13).

Table 13. 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

Upon request, Genzyme submitted information regarding these reversals. A contractor for the dispensing of trial medication kits (-----) failed to write Genzyme-assigned subject identification numbers on first kits (subjects were to receive more than one kit during the trial) for use at sites 5, 6, and 7. Genzyme was notified of the problem and was able to correct it prior to distribution of kits at sites 5 and 6. Correct kits were assigned to subjects 503 and 504; however, site personnel were concerned that the treatments received were incorrect (based on subject 504 being treated before, not after subject 503). Genzyme writes, “The understanding that these two patients were randomized out of sequence led to further communication to the CSU [Genzyme Clinical Supplies Unit] that the kits assigned to patients 0503 and 0504 were switched. Based on this information, the subsequent shipments of investigational product for these 2 patients were based on this reversed treatment allocation error.”

At site 7, distribution was made without subject identification numbers. Allocation appears to have been made without knowledge of the contents of the kits (the unblinding list was not available at the site, and CBER inspection of kit labels shows that identifying information had not been revealed). Table 14 shows that at site 7 four subjects received the correct allocations, while there were 4 misassignments.

Table 14. AGAL-1-002-98: Site 7 treatment allocations

Subject randomization no.	Agent assigned	Kit assigned	kit received per CRF	date of 1st infusion	time of infusion (all a.m.)
701	r-h α Gal	3007	3006 (placebo)	June 11	10:05
703	placebo	3006	3008	June 11	10:28
704	placebo	3008	3012	June 11	10:30
705	placebo	3012	3007 (r-h α Gal)	June 11	10:55
707	r-h α Gal	3010	3009	June 18	10:00
706	placebo	3011	3010 (r-h α Gal)	June 18	10:20
702	r-h α Gal	3009	3015	June 25	9:15
708	r-h α Gal	3015	3011 (placebo)	June 25	9:45
709	placebo	3013	3013	July 9	8:30

Other, more minor dispensing errors were noted in the BLA:

- Subject 801 was erroneously given one treatment from another subject's medication kit, but this happened to be subject 801's correct treatment. The kit was resupplied with a treatment for use by the subject for whom it had been intended (according to a communication with Genzyme).
- Site randomization codes for site 5 were used for site 6 and *vice versa*.

Other protocol deviations

An adequate kidney biopsy sample could not be obtained at the end of the trial for a subject assigned to active treatment (subject 307) and an end-of-trial heart biopsy was not attempted for a placebo subject (0803) due to procedural complications when the baseline sample was taken.

Urinary GL-3 determinations (which were used for the secondary endpoint) at both sites in France were unevaluable: at site 7 baseline urine samples were compromised due to faulty filtration procedures, and at site 8, the centrifugation method rendered the samples unevaluable.

Other protocol deviations occurred, such as deviations from expected times of collection data, informed consent procedure deviations, and failure to collect information relevant to secondary and tertiary endpoints. Renal plasma flow was not assessed due to the unavailability of the reagent used for the test (PAH). These protocol deviations would not be expected to affect the overall judgment of the results.

Comments

Treatment misassignments apparently occurred due to an error that was localized to certain sites and not reflective of overall trial conduct, and they were made without unblinding treatments. Since there is no evidence of a systematic bias in treatment assignments, analysis of the outcomes of these subjects can be considered an "intent-to-treat" analysis. Subjects 503 and 504 can be included, since they received a substantial majority of their infusions (8/11) toward the end of the trial without alternating treatment assignments.

Adherence to dosing

Adherence to trial drug infusion was excellent. When infusion dose is calculated as an average over all infusions per subject, subjects received a dose per kilogram that was within 1/10 of a mg/kg of the protocol-defined limit. All subjects received all of their infusions.

RESULTS: DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Seven persons were screened who did not participate in the trial: three chose not to participate, three would not be candidates for renal biopsy (increased risk for bleeding, an atrophic kidney, and bilateral renal cysts), and one failed to meet biochemical inclusion criteria for endogenous α -Gal activity. Table 15 shows demographics of the enrolled subjects. No major differences between treatment groups are evident. 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).

Table 15. 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	n (%)	29 (100)	27 (93)
Female	n (%)	0 (0)	2 (7)
Race:			
White	n (%)	26(90)	27(93)
Non-White	n (%)	3(10)	2 (7)

Table 16 shows baseline characteristics of the subjects. 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.

Table 16. 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

RESULTS: 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 randomized grouping. This review will focus on this “as-treated/intent-to-treat” population. Genzyme 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

The distribution of baseline scores is seen in Table 17. Based upon Fisher's exact test, there were no differences between the active and the placebo group in the distribution of baseline kidney slide scores. However, there was a weak trend toward greater baseline severity in the placebo group.

Table 17. 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 18 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 subjects 307 in the r-h α Gal-treated group. A nonzero end-of-trial value was scored for each the biopsies of the two subjects (numbers 503 and 504) who received partial treatments, one in each treatment group.

Table 18. Trial AGAL-1-002-98: Primary endpoint results*

End of trial score	Placebo n=29	r-h α Gal n=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 19 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 19. Trial AGAL-1-002-98: Distribution of end-of-trial slide scores*

End of trial score	Placebo n=29	r-h α Gal n=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, nonzero scores were only in the mild category, but that in the placebo group scores were distributed among mild, moderate, and severe. The one end-of-treatment severe score in the active group was an attributed score. The results show that the effect of r-h α Gal was to diminish GL-3 regardless of baseline severity.

Table 20 shows the results of Genzyme's ANOVA, an additional analysis specified in the protocol. The results are consistent with the χ^2 test described above.

Table 20. Trial AGAL-1-002-98: ANOVA on kidney slide scores*

		Placebo n=29	r-h α Gal n=29
Baseline	mean	2.2	1.9
	std.dev.	0.68	0.80
End of trial	mean	2.1	0.4
	std.dev.	0.79	0.68
Change	mean	-0.1	-1.6
	std.dev.	1.13	1.15
difference in means		-1.48	
95% C.I.		(-2.08, 0.88)	
p-value		<0.001	

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

Review of transmission electron microscopy

During the second, quantitative reading of the kidney slides pathologists answered the following question with regards to kidney capillary endothelium: “Does the appearance (quantity, distribution, size, etc.) of inclusions on TEM photomicrographs match your expectations based on your evaluation of the LM [light microscopy] slide for this patient's tissue?” At baseline, 4/29 and 8/29 slides were scored for the placebo and r-h α Gal-treated groups; at the end of the trial, 7/29 and 28/29 were scored, respectively (the larger number of slides examined in the treated group results from the greater number of slides with scores of 0 and 1 that were subject to a re-reading procedure in that group). 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.

Primary endpoint scores for subjects with treatment errors

Table 21 shows that 2 subjects incorrectly given placebo improved, while 1 deteriorated; conversely, 2 subjects incorrectly given r-h α Gal improved, while one stayed the same.

Table 21. Trial AGAL-1-002-98: Primary endpoint scores for subjects having treatment dispensing errors

Patient Number	Blinded Randomization	Treatment Received	Baseline	Visit 11 (Week 20)
503	r-h α Gal	Placebo	2	1
504	Placebo	r-h α Gal	1	1
701	r-h α Gal	Placebo	3	1
705	Placebo	r-h α Gal	3	0
706	Placebo	r-h α Gal	2	0
708	r-h α Gal	Placebo	1	2

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

Table 22 shows the baseline scores of those who ended with a zero score, by treatment group. These data show that the activity of the product is not limited to those with lower baseline kidney slide scores; in fact, the majority of subjects who achieved a score of "0" at the end of treatment had baseline scores of "2" or "3."

Table 22. 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

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. Considering the "as-treated" population, no pathologist scored a "0" for any placebo subject at the end of the trial. Among the active subjects Dr. Rennke scored 21/29 with a score of "0," Dr. Colvin 12/29, and Dr. Dikman 27/29. Thus all pathologists gave the active-treated groups many more "0" scores. The intent-to-treat evaluation gave results consistent with the as-treated evaluation.

Genzyme showed that among the reread slides with a final score of "0" (20 slides, from the intent-to-treat population) the proportion of capillaries with a capillary score of "0" was almost uniformly more than 90% for each pathologist.

An unusual finding from the examination of consistency of scoring was in the discrepancy among the pathologists in the number of capillaries scored. Genzyme showed the capillary counts by pathologist for each slide that was subjected to a rereading (having obtained a score of "0" or in "1" on the initial reading). There was considerable variability, with Dr. Rennke scoring the most capillaries in a given biopsy in almost all cases, sometimes by 100 or more. The exact reason for this is unclear; Genzyme postulates that individual technique played a role as well as field of view of the microscopes used (Dr. Rennke using the widest field of view). However, as each pathologist

was supposed to cover the entire slide, the field of view should not have been an issue. 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.

Sensitivity to slide capillary scoring criterion

Genzyme examined the proportion of "0" scores as a function of the cutoff value used to eliminate outliers from the re-read capillary counting procedure. The analysis used the intent-to-treat population (groups representing the treatment assigned to subjects, regardless of the treatment actually received). When a cutoff value of 1% was used (thus eliminating only 1% of the capillaries with the highest scores), the numbers of subjects with "0" scores at the end of treatment were 10 and 1 in the active and placebo group, respectively. Using a criterion of 10%, which allows more inclusion-bearing capillaries to be discarded in the scoring, the numbers of subjects with "0" scores at the end of treatment in the active and placebo groups is 24 and 3, respectively. Thus a definite advantage of treatment was not critically dependent on the cutoff of 5% chosen for aberrant vessels in the kidney slides. CBER reached the same conclusion performing the same analysis on the as-treated population.

Effects of age on primary endpoint

Genzyme stratified the endpoint analysis at the median age of the overall population, 30 years (the BLA contained an intent-to-treat analysis; an as-treated analysis was provided upon request). The analysis of age is important since Fabry's disease is a congenital disorder; duration of disease is the same as age. Table 23 show 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).

Table 23. 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). None of the 3 non-White placebo subjects ended with a "0" score, while 1 of the 2 non-White r-h α Gal-treated subjects ended with a "0" score. These results are consistent with the overall results, but are of little use since the numbers of subjects is small. Of the two females in the trial, both on active treatment, 1 started and ended with a score of "0" and the other started with "1" and ended with "0." No conclusions can be made regarding gender from this small amount of data.

Effect of trial site on endpoint

Table 24 shows that overall results were generally consistent from site to site. 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. The p-value is based on the Mantel-Haenszel χ^2 test stratified by trial center.

Table 24. Trial AGAL-1-002-98: Numbers of subjects with “0” or “non 0” score at the end of treatment, by trial center*

Site	Treatment Group [n, (%)]				Odds ratio	95% C.I.	p-value
	Placebo		r-h α Gal				
	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

CBER's exploration of the primary endpoint

Table 25 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 25. 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-h α Gal	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 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.

Adequacy of slide samples for localization of affected capillaries

Eight kidney slides contained medullary tissue only (comments indicated medulla tissue only was examined in 7 cases and “probably medulla” in 1). Baseline medullary-only slides occurred in

the r-h α Gal-treated group only; there was a single 1, 2, and 3. The remaining, end-of treatment slides showed a similar treatment effect to that of the overall group (3 placebo slides with scores of 2, 3, and 3 and 2 r-h α Gal slides with 0 scores).

Time to final biopsy

The kinetics of the reappearance of inclusions of GL-3 are unknown. If GL-3 inclusions reappeared rapidly, and there were a bias in the timing of the final biopsies, this could have resulted in a bias in the reported results. Times to biopsies were similar: biopsies occurred up to 22 days after the final infusion in the placebo group and up to 26 days after the final infusion in the r-h α Gal group, with medians of 12 and 11 days, respectively. In addition, there was no correlation between the change in score and the time to biopsy for either group ($r = -0.04, p=0.8$ and $r = 0.2, p=0.3$, respectively). The analysis excluded subject 307, who didn't have a final biopsy.

Subgroup analysis of the primary endpoint (age)

Table 26 shows the proportion of r-h α Gal-treated subjects who did not achieve a "0" score at the end of the trial, by baseline score. 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. The distribution of baseline scores in the two age groups is similar. Whether this distribution of severities would hold for a large sample is an open question.

Table 26. Trial AGAL -1-002-98: Numbers of subjects who failed to meet the endpoint criterion, by baseline score and age (r-haGal-treated subjects)

Baseline score	Number of subjects <30 yrs.	number not achieving "0"	Baseline score	Number of subjects 30+ yrs.	number not achieving "0"
0	1	-	0	0	-
1	1*	1	1	5	3
2	5	0	2	9	2
3	3	1	3	4	2
total	10*	2	total	18	7

*omits subject 307, attributed a worst case score due to missing sample

CBER also examined the distribution of change scores in the r-h α Gal group when categorized by quartile of age. There was no age-related pattern in the distribution of change scores.

Table 27. AGAL -1-002-98: Change Score by age in r-haGal-treated subjects

Age	Change Score					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 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 review).

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 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.” A review of Dr. Jennette’s slide scores shows a very good correspondence to the scores given by the renal pathologists trained and employed by Genzyme (Table 28).

Table 28. 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.

Comments on primary endpoint

The activity of r-haGal 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. The overall results were not driven by site, but occurred nearly uniformly throughout the trial.

Secondary endpoint: McGill pain questionnaire

The Short Form McGill pain questionnaire contains 15 questions relating to pain, divided into sensory or affective groups. Each question is rated on an intensity scale as 0=none, 1=mild, 2=moderate, and 3=severe. The questionnaire includes a visual analogue scale (VAS) consisting of a straight line anchored on the ends with the words “no pain” and “worst possible pain” and a question on present pain intensity (PPI) in which the subject chooses among 6 descriptors ranging from “no pain” to “excruciating.” The statistical plan called for examining the change from baseline to the end of the trial for sensory and affective groups of questions and for the total of the 15 sensory and affective questions. In addition it called for analysis of the change from baseline for the VAS and for the present pain intensity question. The statistical plan called for an analysis to be performed “similarly” to that of the primary endpoint. Importantly, subjects were not restricted in their use of pain medications during the trial.

Table 29 shows the results of the Short Form 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.

Table 29. Trial AGAL -1-002-98: Short Form McGill pain questionnaire results*

Pain measure		Placebo	r-h α Gal
Sensory pain	baseline mean \pm s. dev.	4.7 \pm 6.1	5.7 \pm 6.9
	mean change \pm s. dev.	-3.2 \pm 6.5	-2.9 \pm 6.8
	Intergroup difference in mean change \pm s.dev.	0.31 \pm 0.31	
	95% C. I.	(-3.18, 3.80)	
	p-value	0.86	
Affective pain	baseline mean \pm s. dev.	1.7 \pm 2.5	1.8 \pm 2.1
	mean change \pm s. dev.	-1.4 \pm 2.6	-1.0 \pm 2.0
	Intergroup difference in mean change \pm s.dev.	0.41 \pm 0.60	
	95% C. I.	(-0.79, 1.62)	
	p-value	0.50	
Total pain	baseline mean \pm s. dev.	6.4 \pm 8.2	7.4 \pm 8.5
	mean change \pm s. dev.	-4.7 \pm 8.6	-3.9 \pm 8.4
	Intergroup difference in mean change \pm s.dev.	0.72 \pm 2.23	
	95% C. I.	(-3.74, 5.19)	
	p-value	0.75	
PPI	baseline mean \pm s. dev.	1.2 \pm 1.2	1.4 \pm 1.3
	mean change \pm s. dev.	-0.5 \pm 1.3	-0.7 \pm 1.7
	Intergroup difference in mean change \pm s.dev.	-0.17 \pm 0.39	
	95% C. I.	(-0.96, 0.62)	
	p-value	0.66	
VAS	baseline mean \pm s. dev.	2.1 \pm 2.4	2.4 \pm 2.5
	mean change \pm s. dev.	-1.4 \pm 2.4	-1.0 \pm 2.7
	Intergroup difference in mean change \pm s.dev.	0.44 \pm 0.67	
	95% C. I.	(-0.91, 1.79)	
	p-value	0.51	

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

Subject diaries, which include pain medication usage, are 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

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. Table 30 shows the baseline scores for the skin and heart. The distributions were similar between the treatment groups.

Table 30. 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 31 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 31. 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 n=29	r-h α Gal n=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

These majority scores were added to that of the kidney to create a composite score. Table 32 shows the differences in means, as well as the results of the t-test on the means, for each component of the endpoint. It is important to note that the composite score does not have fully independent meaning from the primary endpoint, as the primary is contained within it.

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.

Table 32. Trial AGAL -1-002-98: Heart, skin, and kidney biopsy results with composite score*

		Placebo n=29	r-h α Gal n=29
Heart	baseline mean	0.9	0.9
	mean change \pm s. dev.	0.2 \pm 0.8	-0.6 \pm 0.7
	difference in means	-0.79	
	95% C.I.	(-1.19, -0.39)	
	p-value	<0.001	
Skin	baseline mean	2.3	2.1
	mean change \pm s. dev.	-0.1 \pm 1.0	-2.1 \pm 0.7
	difference in means	-2.03	
	95% C.I.	(-2.49, -1.58)	
	p-value	<0.001	
Kidney	baseline mean	2.2	1.9
	mean change \pm s. dev.	-0.1 \pm 1.1	-1.6 \pm 1.2
	difference in means	-1.48	
	95% C.I.	(-2.08, -0.88)	
	p-value	<0.001	
Composite	baseline mean	5.4	4.9
	mean change \pm s. dev.	0.1 \pm 2.1	-4.2 \pm 1.8
	difference in means	-4.31	
	95% C. 1.	(-5.33, -3.29)	
	p-value	<0.001	

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

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 33 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 33. 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-haGal.

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-haGal 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 33. 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. The results will be briefly summarized. Some of the measures were assessed in U.S. subjects only; 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. Twenty-five levels of stimulus threshold, where 13 is normal. At baseline, patients in the active-treatment ($n=16$) and placebo treatment ($n=14$) groups had similar scores (levels 16 and 15, respectively). There was no difference in the change from baseline observed between treatment groups at Visit 11 (mean values of 15.9 and 16.7, respectively).
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. This review will analyze the summary data provided by Genzyme, which sums the scores obtained from right and left sides of each subject. Sensation baseline scores were similar (placebo and active mean 0.2 and 0.3, respectively, with a nominal maximum of 32) and changes were similar (placebo and active means of -0.2 and -0.1 , respectively). Cranial nerve mean scores were 0 for both groups and didn't change in either group. Muscle weakness scores were low in both groups at baseline (mean of 0.2 and 0.9, respectively, with a nominal maximum of 152); changes at the end of the trial were similar (-0.2 and 1.1, respectively). Reflex scores were similar at baseline (0.5 and 0.6 respectively, with a nominal maximum of 40) with similar changes at the end of the trial (mean changes of -0.5 and -0.4). Lower limb baseline scores were similar (placebo and active means of 0.4 and 1.4, with a nominal maximum of 32) and changes were similar at the end of the trial (placebo and active means of 0.4 and 1.4 and -0.4 and 0.9, with a nominal maximum of 32). 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. "Symptom," "severity," and "change" scores were obtained at baseline and end of trial in the following categories: head and neck, chest, upper limb, lower limb, overall weakness, sensory, autonomic, and total, yielding 24 sets of scores. Baseline scores were rarely over 2, the minimum score that denotes neurological impairment, and the changes were also small throughout. 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, calculated for a total of 55 patients. The range of scores for the TSS (comprised of four symptoms: stabbing pain, burning, prickling/pins and needles, and numbness/feeling of being asleep) is from 0 to 14.64 (all four symptoms present, severe, and continuous). At baseline in both treatment groups the score was low (as-treated groups placebo 1.7 ($n=43$) and r-h α Gal 2.5 ($n=45$)). Mean scores for both groups fell by less than a point in both groups (end-of-treatment scores in as-treated groups: placebo, 1.0 ($n=43$) and r-h α Gal, 1.4 ($n=45$)).
5. SF-36 Health Status Survey, measured in all subjects. 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). Scores range from a nominal 0 (poorest score) to 100 (best health). Table 34 shows that baseline scores were similar for all subscales except for role limitations due to physical function; end-of-treatment scores for subscales were almost identical. Genzyme does not provide a comment on the clinical meaning of any increments in scores.

Table 34. Trial AGAL -1-002-98: Mean scores on SF-36 questionnaire (mean \pm std. error)*

	Placebo		r-h α Gal	
	Baseline	Visit 11/13	Baseline	Visit 11/13
<i>n</i>	29	29	29	29**
Physical functioning	70.5 \pm 4.1	76.0 \pm 4.0	63.4 \pm 4.6	67.9 \pm 5.0
role limitations due to physical function	43.1 \pm 7.4	59.5 \pm 8.2	39.7 \pm 6.9	58.6 \pm 7.3
body pain	49.8 \pm 4.3	64.7 \pm 4.2	52.8 \pm 4.2	59.4 \pm 3.6
general health	53.7 \pm 2.4	53.6 \pm 1.9	53.1 \pm 2	54.4 \pm 1.7
vitality	56.4 \pm 1.8	57.4 \pm 1.6	57.6 \pm 2.3	56.4 \pm 2.2
social functioning	48.7 \pm 2.2	48.3 \pm 1.5	50.0 \pm 1.8	46.6 \pm 1.9
role limitations due to emotional function	72.4 \pm 7.0	70.1 \pm 7.1	55.2 \pm 8.2	70.1 \pm 7.6
mental health	65 \pm 1.5	64.9 \pm 1.3	62.3 \pm 2.0	65.4 \pm 1.6
Physical component scale	39.5 \pm 1.8	44.4 \pm 1.7	39.5 \pm 1.7	42.6 \pm 1.7
mental component scale	45.5 \pm 1.1	43.4 \pm 0.9	43.5 \pm 1.2	44 \pm 1.2

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

**n=29 for all except 28 for gh, pcs, and mcs

6. Physician Assessment of Fabry Symptoms and global perception of subject status, measured in all subjects. 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 (as-treated groups, placebo vs. r-h α Gal: 1 vs. 0; 2 vs. 3; and 7 for both, respectively). Three subjects in each treatment group did not have abnormal sweating at baseline; at the end of the trial there was a very small difference of 1 and 3 additional subjects without abnormal sweating in the placebo and r-h α Gal-treated groups, respectively. 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, 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. 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), measured in all subjects. This measure trended toward lower values in the placebo group (as-treated groups: placebo vs. r-h α Gal mean \pm standard deviation, 43 \pm 53 vs. 48 \pm 56).

9. Mean pain score on 0-10 scale, measured in all subjects. 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. The analysis presented by Genzyme (see Table 55, which combines data from AGAL-1-002-98 and its extension, AGAL-005-99) must be interpreted with caution: As a result of difficulties in obtaining inulin after baseline determinations were made, some subjects did not have end-of-treatment inulin tests as planned; for these subjects, GFR was estimated from creatinine clearance by multiplication by 0.77. In addition, baseline glomerular filtration rate was not balanced between the treatment groups, and end-of-trial calculations were performed in a subset of the trial population. GFR improved for the placebo as well as the r-h α Gal-treated group, which is a medically implausible result.

CBER's analysis of subjects with inulin-based GFRs only (Table 56) corroborates the general findings of Genzyme's analysis, although it too must be interpreted with caution due to the nonrandomized nature of this subset.

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. Baseline mean values were slightly different (placebo vs. r-h α Gal means and standard deviations 1.3 ± 0.51 vs. 1.7 ± 0.76), but visit 11 mean values were the same (1.2 ± 0.46 vs. 1.2 ± 0.76). These results are inconclusive and may represent a regression toward a mean (nontreated) value.

Comment

CBER examined serum creatinines as a measure of renal function (see Table 57, which combines AGAL-1-002-98 and AGAL-005-99 results). The results cannot be interpreted to show any treatment effect.

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). Baseline values for the TDT and VOP were similar and not abnormal; the changes to end of the trial were similar. The end-of-trial change in QSART favored the r-h α Gal group (placebo vs. r-h α Gal, -6.8 vs. 13.5 mol/min); however, the baseline values were not comparable (medians of 20.9 and 10.9 mol/min for the placebo and r-h α Gal groups). The reliability of these differences is not clear.

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.

“Other” endpoints

1. Ophthalmological examination findings, using fundus photography if differences were observed. Genzyme reports that no differences were discerned between the treatment groups upon visual examination, and fundus photographs were not examined.
2. Urinary protein to creatinine ratio. Genzyme does not provide an analysis as 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, PAH.
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 statistically significant 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). Table 35 shows that median percents change were not different between treatment groups but there was a difference in changes in group means and median values. 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. There was a difference in the change in heart mean 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.

Table 35. 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
	mean \pm std. error	10796 \pm 1815	9949 \pm 1634		11024 \pm 1740	8850 \pm 1554	
Skin	<i>n</i>	29	28		26	26	
	median	385	343	-24	362	189	-20
	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

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

Genzyme assayed IgE and IgG antibodies to r-h α Gal during the trial. No subject developed IgE against α -Gal A. 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. The earliest time to development of an anti- α -gal IgG was visit 3, the latest, visit 11, with a median and mean time of 42 and 57 days. Time to development of any titer is shown in Table 36.

Table 36. 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.

A subject from the placebo group developed a persistent antibody titer starting at visit 5. Genzyme explored this event, and determined that the subject had not received the active agent during the trial; an explanation for the event is not clear.

Effect on efficacy

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.

CBER analysis of the effect of IgG seroconversion on bioactivity

- Effect of development of any titer

CBER examined the data for a possible correlation between the time to development of any IgG titer and change scores (differences from baseline). Table 37 shows an analysis of the amount of change in renal slide score by time of development of IgG antibodies (antibodies persisted in all subjects to the end of the trial). The analysis shows that the amount of change is not limited by early development of antibodies.

Table 37. Trial AGAL-1-002-98: Change from baseline as a function of visit number at which IgG seroconversion was noted (r-haGal 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

- Effect of level of peak titer

A similar analysis of the change score as a function of peak titer shows that the peak titer did not correlate with the change score developed by individuals (Table 38). Note that this analysis is tentative, since Genzyme's method for titrating antibody was not qualified.

Table 38. Trial AGAL-1-002-98: Change from baseline as a function of the peak titer (r-haGal 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

Pharmacokinetics

For a detailed review of the pharmacokinetics substudy, see the review of the clinical pharmacologist. Samples were taken for pharmacokinetic analysis at infusions 1, 3, 7, and 11 (end of trial). 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. In the few individuals with available data, the area under the curve of serum concentration was reduced by the 11th infusion to 27% in a subgroup of 3 subjects with the highest antibody titers (1:12,800); maximal concentration fell to 26%.

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-haGal.

Summary (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.

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

RESULTS: SAFETY

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. Mean exposure duration for this population was 161 days (standard deviation, 10 days).

Genzyme's r-h α Gal-treated group excluded subject 503 and included subject 504. Genzyme submits a summary of the safety experience of these two subjects during their first 3 infusions. 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 events:
 - 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 39 shows the numbers of subjects with adverse events that occurred in at least 2 more subjects in the active group than in the placebo group, with the severities of the adverse events. 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 39. 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.

Most of the increase in events in the r-h α Gal-treated group occurred in the “mild” category. 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. The great majority (33/40) 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. There were no reports of fever on the day of infusion among the placebo subjects, and rigors occurred once after infusion, about 6 hours afterwards.

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).

Skeletal pain was reported only among r-h α Gal-treated subjects. Among the 6 subjects 8 events (discomfort in the neck, shoulder, and face) were recorded lasting from 11 minutes to about 15 hours. Given the number of events and the distribution of severity, this discrepancy does not appear clinically important. 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 39 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.

There was an increase in dependent edema events, mostly of mild severity, in r-h α Gal-treated subjects. Peripheral edema is a finding in Fabry's disease, but CBER looked for evidence of an adverse effect on cardiovascular function by examining listings of adverse events coded under “cardiovascular disorders.” There was no noteworthy concern from this examination.

CBER's review showed that of the 14/17 hematuria adverse events occurred proximally to the renal biopsy procedure. Of the remaining 3, 1 was in placebo, and 2 in the active-treated group.

Of the eye abnormalities, 2/3 in the r-h α Gal-treated group and 1/1 in the placebo-treated group were noted before the first infusion. The 1 subject with eye abnormalities noted during the trial, in the r-h α Gal-treated group, had "tiny" precipitates behind Descemet's membrane (the posterior membrane of the cornea). Data are insufficient to discern if this finding is due to Fabry's disease.

Infusion of a foreign protein raises the concern for serum sickness. No subject had serum sickness, glomerulonephritis, or vasculitis as an adverse event. The proportions of subjects with albuminuria (a possible sign of nephritis, but seen in Fabry's disease as well) was equal between the treatment groups. Myalgia and arthritides may be sentinel signs of serum sickness. There was no difference in the numbers of subjects with "myalgia" as a preferred term in placebo and r-h α Gal treatment groups (4 and 3 respectively). Data listings were searched for preferred terms with "arth" (this included arthrosis, arthropathy, and arthralgia). There was a moderate imbalance of events, with 1 subject in placebo having knee pain and 4 in r-h α Gal treatment having stiff joints, rigid joints, stabbing pain in joints of fingers, and stiff neck (1 event may be clearly omitted from each group: knee crepitus in a placebo subject and ankle sprain in an r-h α Gal subject). Overall, these data do not suggest that serum sickness occurred.

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. Table 40 shows infusion-related adverse events as syndromes; an occurrence of a single event any number of times in a single subject is counted once, and subjects may appear in more than one category. Most of the 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 40. Trial AGAL -1-002-98: Infusion-related adverse events (r-haGal-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

Investigators were to notify Genzyme Pharmacovigilance in the event of a suspected hypersensitivity reaction. Table 41 shows clinical details of these events, which occurred in 12 subjects (infusion-related events in the 4 remaining subjects were: chills (subjects 301 and 702), headache (subject 307), and abdominal pain (subject 804).

The events in Table 41 occurred at the 4th infusion or later. These events 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.

Table 41. 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 spontaneously 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

In addition to the subjects above, listings show that subjects 105 and 106 had infusion rate changes.

Genzyme Pharmacovigilance was to initiate testing for complement activation (postinfusion serum sample) and serum IgE against r-h α Gal (preinfusion serum sample) for infusion reactions reported to it. Table 42 shows the results of the testing for the presence of anti-r-h α Gal IgE and IgG and complement activation in the subjects detailed in Table 41. Anti-IgG antibody was present in

almost all of these subjects, consistent with the overall high seroconversion rate; however, subject 202 had infusion reactions in the absence of detected IgG antibody. Although IgE was not tested for every reaction, serum IgE was not found in the great majority of subjects at the last infusion tested. 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 (data not shown here).

Table 42. Trial AGAL-1-002-98: Anti α -Gal IgE and IgG and complement activation in association with the events in Table 41

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

Comment

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

Hypertension and product impurity

Three subjects were reported to have moderate hypertension in association with infusion. Genzyme has tentatively identified a hamster angiotensinogen in the product used in both FB9702-01 and AGAL-1-002-98. The tentative identification is based on homology to rat and mouse angiotensinogens. It is possible that human renin could use this angiotensinogen as a substrate, generating angiotensin I. Angiotensin and its metabolite, angiotensin II, can raise blood pressure. The upper limits of angiotensin I that were generated by the material used in FB9702-01 and AGAL-1-002-98 (by an assay using porcine renin, which converts almost completely) were 3.05 ± 0.17 ng/mg and 2.18 ± 0.13 ng/mg, respectively. Based upon comparison of these levels to levels of angiotensin I and II studied in published literature summarized by Genzyme, it is possible that the

infusion of r-h α Gal could account for some of the blood pressure elevations noted during infusion. However, a long lasting effect of these infusions on blood pressure is unlikely.

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 a-Gal, anti-a-Gal IgG, and anti-a-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). Angiotensinogen is not likely to account for episodes lasting days, such as those observed in FB9702-01.

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

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 (not shown) revealed no treatment-related trend.

Comment

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

Concomitant medication use

Data listings were examined for trends in medication usage that might signal safety concern. Table 43 shows numbers of subjects who took various classes of medications for the prominent Fabry symptoms of pain and acroparesthesia, separated by use before the 1st infusion and after (including the day of infusion), and including those medication classes used by 4 or more subjects in either group. Although the time period before infusion 1 was not specified, it appears that medication usage was similar in the two groups.

Table 43. Trial AGAL-1-002-98: Subjects with use of medication for pain or acroparesthesias, before and after the first infusion (among drug use by 4 or more subjects in either group)

Indication	Drug class	Placebo		r-h α Gal	
		Before infusion 1	After infusion 1	Before infusion 1	After infusion 1
pain	anilides	16	18	17	18
	natural opium alkaloids	2	3	1	4
	opium alkaloids and derivatives		1	1	4
	propionic acid derivatives	1	6	2	9
acroparesthesia	carboxamide derivatives	0	5	1	2

Examination of other medications shows that 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

For information on the rates of seroconversion and times to seroconversion, refer to the section on antibody development in the efficacy review. Genzyme tabulated the adverse events occurring in 2 or more active-treated subjects either among those who developed an IgG antibody titer (n=24) or among those who did not (n=5). Numbers of subjects in the latter group make comparisons tenuous; 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 verifiable as that for the kidney (because they were not based on a method using quantitation of slide parameters), it appears that they showed 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.

COMMENTS ON THE PRIMARY ENDPOINT

The exact mechanism leading to the pathologic physiology of Fabry's disease is not as well-defined as the primary enzyme defect. However, there is widespread belief that a number of the organ injury manifestations are related to vascular injury. It is believed that while this may not be the sole pathologic process, progressive substrate accumulation within vascular walls will ultimately lead to local vessel occlusion, with organ impairment as a consequence.

Vascular injury does appear to be an important mechanism promoting progressive organ impairment, and substrate accumulation within vascular walls is the basis for this. The exact (quantitative) relationship between the amount of substrate accumulation and the degree or rate of vascular ischemia is unknown and not addressed in any information submitted by Genzyme. It is unknown if reducing substrate accumulation by half, for example, might slow vascular injury by half, or if there is a threshold effect, whereby some specific amount of accumulation will invariably lead to vascular occlusion and thus no change in the clinical expression of the disease. However, by focusing upon a near-elimination of all accumulation within a specific cell type Genzyme's data appear to overcome these concerns. Genzyme has shown that capillary endothelium is altered by the

enzyme treatment to achieve a near-normal appearance with regard to accumulation. Vessels (capillaries in this case) that are essentially near-normal in appearance may well lead to an altered development of vascular occlusion, and thus to an alteration in expression of the clinical impairments of the disease. At a public meeting in January, 2003 an Advisory Committee supported this assessment of the potential impact of near-absence of capillary accumulation, as well as concurring that the evidence submitted by Genzyme have demonstrated this effect on capillary endothelium.

Consequently, FDA has determined that Genzyme has shown an effect upon a surrogate endpoint reasonably likely to predict a clinical benefit.

TRIAL: AGAL-005-99

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

Six-month results

DESIGN

This trial is an ongoing extension to AGAL-1-002-98. 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 it. Both groups are to receive r-h α Gal at the dose chosen for AGAL-1-002-98. The duration of the trial was projected as 18 months or until market approval. The trial's objectives are to determine safety and to ascertain bioactivity primarily by means of kidney, skin, and heart biopsies.

Comment on the design of trial

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

Treatment and concomitant medications

Subjects were expected to enter the trial approximately 2-6 weeks after the last infusion in AGAL-1-002-98. All subjects were to be treated every 2 weeks with the same dose of r-h α Gal (0.9-1.1 mg/kg) that was used in that trial. Pretreatment for infusion reactions was to be the same as in AGAL-1-002-98, that is, acetaminophen 975 to 1000 mg, and hydroxyzine 25 to 50 mg, orally. After about 4 months of involvement in AGAL-005-99, subjects were to be allowed to receive further infusions at a local site.

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), including clinically significant cardiovascular, hepatic, 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

Procedures and evaluations

Subjects were to enroll in AGAL-005-99 upon completion of AGAL-1-002-98. The protocol called for biopsies of skin at 6 and 12 months (18 month biopsy optional); Biopsy of the kidney and heart were to be performed at month 6 only, but could be postponed to month 12.

The following summarizes additional procedures and evaluations in relation to the entry infusion to AGAL-005-99:

- Every 2 weeks (starting at entry):
 - Infusion of r-h α Gal
 - vital signs
 - adverse event assessment and concomitant medications
 - serum antibody to r-h α Gal (every 2 weeks for the first 6 months only, then every 3 months)
- Entry and month 3
 - physical examination and vital signs
 - McGill short form Pain Questionnaire, SF-36 Health Survey, Fabry Symptom Assessment, Total Symptom Score questionnaires
- Entry, month 6, month 12, and month 18 except as noted:
 - physical examination
 - ECG
 - plasma and 24-hour urinary GL-3 level
 - echocardiogram (not at month 12)
 - clinical chemistry and hematology, urinalysis
 - McGill short form Pain Questionnaire, SF-36 Health Survey, Fabry Symptom Assessment, Total Symptom Score questionnaires
 - ophthalmic evaluation (month 12 only)
 - Renal function testing (inulin, PAH, or urea and creatinine clearance)
 - Neurophysiology Function Test (U.S. subjects only; not month 12)
- In United States subjects only:
 - Gastrointestinal Assessment questionnaire at entry and every 4 weeks

The protocol stipulated that after 8 infusions subjects could receive additional infusions and assessments at a local facility, but that the 6, 12, and 18 month assessments should occur at the AGAL-1-002-98 trial site.

Notes on entry evaluations

Echocardiogram, heart, kidney, and skin biopsy, neurophysiological function testing (U.S. patients only), ophthalmic examination, and PAH and inulin clearance or urea and creatinine clearance tests from AGAL-1-002-98 were not required to be repeated prior to entry into the trial. However, if ECG, safety laboratory tests (blood and urine), r-h α GAL serum antibody assay, and plasma GL-3 were not done within 28 days of the first infusion they were expected to be redone. If vital signs, physical examination, 24-hour urinary GL-3, questionnaires, or urine pregnancy test were not done within 14 days of the first infusion, they were expected to be redone.

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.

Efficacy measurements

Following is the list of endpoints from the protocol, in the order presented. Only the gastrointestinal questionnaire was new compared to AGAL-1-002-98. The statistical analytical plan, made final on July 18, 2000, stated that the histological appearance of the kidney was the primary efficacy measurement.

- morphologic assessment of GL-3 inclusions in the capillary endothelium (vasculature) of the kidney
- changes in McGill Pain Questionnaire (short form)
- changes in autonomic status as measured by a composite score of :
 - Quantitative Sudomotor Axon Test (QSART)
 - Thermal Detection Threshold
 - venous occlusion plethysmography
- change in glomerular filtration rate as assessed by inulin or urea and creatinine clearance
- change in GL-3 level using a composite score of the kidney tissue and urine levels assayed by ELISA
- change in composite score of GL-3 inclusions in the capillary endothelium (vasculature) of the kidney, skin, and heart as measured by light microscopy
- assessment of urinary protein excretion by quantitation of urinary albumin excretion, total protein excretion, and fractional clearance of β 2-microglobulin, normalized using urinary creatinine measures and obtaining simultaneous serum determinations
- ophthalmic changes
- change in Vibration Detection Threshold
- change in SF-36 Health Survey Scores
- change in Gastrointestinal Questionnaire (in U.S. patients only)
- change in Total Symptom Score
- change in physician assessment of Fabry symptoms

Analysis of endpoints

The pathological analysis of kidney slides was specified in the statistical analytical plan made final on July 18, 2000, and the method for scoring slides codified in a pathologist's training manual dated July 21, 2000. Kidney biopsy slides were to be read by 3 independent pathologists, with each evaluable capillary scored on a severity scale from 0-3. The final kidney biopsy score was to be determined using the following rules:

- The highest 5% of capillary scores were to be discarded from analysis of each slide, and the remaining capillaries (95% of the total number) were used for determination of the slide score as follows:
 - A "0" was to be scored as in AGAL-1-002-98, that is, more than 50% (of the remaining 95%) of vessels were to be clear with the others clear of inclusions or with trace amounts of inclusions.
- Remaining slides were scored as follows:
- A "1" was to be scored if 50% or more (of the remaining 95%) of vessels were 0, trace, or 1
 - A "2" was to be scored if 50% or more (of the remaining 95%) of vessels was 0, trace, 1, or 2

- A "3" was to be scored if 50% or more (of the remaining 95%) of vessels was 0, trace, 1, 2, or 3

A majority score would be determined; if a majority did not exist, then the median score would be used.

The pathological analysis of heart and skin biopsy slides was qualitative, as in AGAL-1-002-98. In the case of discrepancies among the pathologists of greater than a point, the slides were to be reread and adjudicated. If an adjudicated score could not be agreed upon, the median score would be assigned.

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.

Comments

The slide score definitions of 1, 2, and 3 were imprecise. For example, slides with a score of "1" or "2" could theoretically each have 47.5% (50% of 95%) capillaries with grade 3 inclusions. However, the slide score of "0" is the same as that in AGAL-1-002-98, allowing an assessment of the occurrence of total or near total reduction among those switched to r-haGal, and persistence of effect in those continued on r-haGal.

RESULTS: DATA SUBMITTED

Biopsy and bioactivity data are reported for up to infusion 14. A maximum of 1 year of safety data were submitted: nonserious adverse event data to infusion 14 (the latest date approximately July 15, 2000), and serious adverse event data up to August 31, 2000.

RESULTS: CONDUCT OF TRIAL

Dates of the trial

The trial started in September, 1999 and is ongoing.

Formal protocol modifications

The original protocol was dated August 19, 1999. It was amended once, on February 25, 2000. This protocol amendment consisted of mostly minor changes, the most important being the addition of tests of renal function through urinary testing and the specification that an adjudication procedure would be performed in case of discrepant scoring among the renal pathologists. In addition, the statistical analytical plan for the 6-month interim analysis was made final on July 18, 2000, and the method for scoring slides codified in a pathologist training manual dated July 21, 2000.

Enrollment and adherence to infusion schedule and dose

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

Genzyme had the expectation that subjects would enter AGAL-005-99 approximately 2-6 weeks after the last infusion in AGAL-1-002-98; as mentioned before, the mean, not maximum, time to entry to the extension was 45 days for each group (from 6-161 days after completion of AGAL-1-002-98; mean and median time to first infusion did not differ notably between the treatment groups). Adherence to the infusion schedule was excellent. At visit 14, the number of subjects receiving infusions for the placebo and r-haGal groups was 27 and 28; prior to that, the smallest number of subjects receiving an infusion at any given visit was 25. Adherence to the protocol-defined dose was excellent, and fell within the same tolerances as discussed above for AGAL-1-002-98.

Discontinuation

Subject 304, previously treated with placebo in AGAL-1-002-98, was withdrawn from AGAL-005-99 because of hypersensitivity symptoms and a reaction to skin testing with r-h α Gal after 8 infusions of r-h α Gal.

Protocol violations

Genzyme acknowledges that “numerous” protocol violations were recorded. These include 35 violations of biopsy procedures, mostly recorded as biopsies not performed or not performed at visit 14. Biopsy violations are not all identified by organ type; if one assumes that biopsies not identified by type are all kidney (the primary endpoint of AGAL-1-002-98), 4 violations of kidney biopsy timing occurred in AGAL-005-99 in addition to 7 biopsies recorded as not done in the placebo/r-h α Gal group, and 4 biopsies not done in the r-h α Gal/r-h α Gal group.

There were 68 infusion violations, 31 in which r-h α Gal was not given, and 38 in which it was not given within the protocol-defined time window. Both types of violation were nearly equally distributed between the treatment groups.

There were 46 violations of the collection of 24-hour urines (used for substantiation of the effect of r-h α Gal on renal function): 30 urines were not performed at visit 1 or 14 (nearly equally distributed between the treatment groups), 4 urines were filtered late (equally distributed), and 5 were collected late (nearly equally distributed). All urines for site 8 (3 subjects in each treatment group) were collected over 20, not 24 hours.

There was a small number of subjects with inulin tests (for GFR, as substantiation of a clinical effect) taken outside the window, equal in each treatment group (4 in the placebo/r-h α Gal group and 3 in the r-h α Gal/r-h α Gal group).

Other violations, including minor informed consent violations and violations of the collection of ECGs, echocardiograms, Fabry symptom score, lab tests, physical exams, quality of life questionnaire data, and vital signs occurred.

Comments

The data on biopsies reviewed above does not suggest that the number of subjects who failed to get biopsies would have had an important effect on the overall conclusions. There were few kidney biopsies that took place outside the protocol time window.

Infusion violations were numerous, but not overwhelming in relation to the nominal number of infusions given (58 x 14=812).

Violations of the performance of 24-hour urine collections were common (a nominal 58 subjects with 2 urine collections, or 106 nominal urine collections), making use of these data generated from these collections problematic.

RESULTS: BASELINE CHARACTERISTICS

The subjects in AGAL-005-99 were the same subjects as participated in AGAL-1-002-98 (see Table 15 and Table 16).

RESULTS: EFFICACY

Efficacy results will be reviewed in parallel with those of AGAL-1-002-98, and not in the order that they were presented in the extension protocol. Results are shown for the as-treated group from AGAL-1-002-98.

Completeness of data

Table 44 shows numbers of subjects with 6-month biopsy data reviewed here. The majority of subjects were available for most biopsies.

Table 44. AGAL-005-99: Subjects (% of group) with 6-month biopsies

Organ	Placebo/r-h α Gal n=29	r-h α Gal /r-h α Gal n=29	Total n=58
Kidney	24(83)	25 (86)	49(85)
Heart	18(62)	22(76)	40(69)
Skin	26(90)	27(93)	53(91)

The same pathologists who participated in AGAL-1-002-98 read the biopsy slides for this trial.

Primary endpoint: GL-3 in kidney biopsy

Table 45 shows the kidney biopsy slide entry score for AGAL-005-99, separated by treatment group. These are the end-of-treatment scores from AGAL-1-002-98.

Table 45. AGAL-005-99: Kidney biopsy entry scores (same as end-of-treatment score in AGAL-1 -002-98)*

Entry score	Placebo/r-h α Gal n=24	r-h α GAL /r-h α Gal n=24
0	0	18
1	5	6
2	9	0
3	10	0

*Only in subjects with 6-month AGAL-005-99 biopsies listed; excludes subject 307, who did not get an end-of-trial biopsy in AGAL-1-002-98

Table 46 shows results expressed as zero score at 6 months, separated by treatment group.. These data show that all placebo subjects tested (22 of 29 from AGAL-1-002-98, none of whom had a 0 score at entry to the trial) had a score of 0 at 6 months of AGAL-005-99. All subjects but 1 (previously treated with r-h α Gal in AGAL-1-002-98 had a 0 score at 6 months of this extension trial (score went to 1). This latter finding is important, since it shows that the effect on substrate in renal capillary endothelium does not disappear with 6 months of additional treatment.

Table 46. AGAL-005-99: Score of "0" on kidney biopsy at 6 months

Treatment Group	Score at entry to AGAL-005-99	6-Month Post Entry Score		Total
		Zero	Nonzero	
Placebo/ r-h α Gal	Zero	0	0	0
	Nonzero	24	0	24
R-h α Gal / r-h α Gal	Zero	17	1	18
	Nonzero	6	0	6

The analysis omits a single subject (subject 307, r-h α Gal) due to a missing biopsy at visit 11 of AGAL-1-002-98 ("entry" value)

Genzyme's exploration of primary endpoint

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

Consistency of the pathologists' scoring

Table 47 shows that the pathologists' ratings of "0" and "non-0" were very consistent.

Table 47. AGAL-005-99: Zero and nonzero scores attributed to kidney pathology biopsies by individual pathologists*

Entry score	6-month score											
	placebo/r-h α Gal n=22						r-h α Gal/r-h α Gal n=21					
	Rennke		Colvin		Dikman		Rennke		Colvin		Dikman	
	0	Non-0	0	Non-0	0	Non-0	0	Non-0	0	Non-0	0	Non-0
0	0	0	0	0	0	0	18	1	10	1	22	1
non-0	23	1	18	6	24	0	5	0	10	3	1	0

The analysis omits a single subject (subject 307, r-h α Gal) due to a missing biopsy at visit 11 of AGAL-1-002-98 ("entry" value)

Effect of change in criterion for aberrant vessels

Genzyme analyzed 6-month "0" and "non-0" scores as a function of the proportions of capillaries disregarded in the slide scoring. This analysis was performed on a selection of kidney biopsy slides available around the time of the initial BLA submission (22 subjects in each treatment group, the great majority of the subjects presented here). Table 48 shows the extremes of analysis of the application of criterion values for the discounting of capillaries at both the end of AGAL-1-002-98 and at 6 months of AGAL-005-99; intermediate values in the analysis are consistent with the ones shown here. As Genzyme points out, data from low-criterion-value (high-stringency) rows suggest that there is a continued treatment effect after 6 months. That is, among subjects who entered the extension with a non-0 score based on the higher, 1% stringency of the capillary-disregarding criterion, the majority reached the 6-month time point with a 0 score. Data from the higher-criterion-value rows (10% shown) are unable to discriminate this effect, as nearly everyone entered the extension with a 0 score.

Table 48. AGAL-005-99: Zero and nonzero status at the 6-month time point as a function of criterion value for the discarding of aberrant values in computation of kidney biopsy status at the end of AGAL-1-002-98

Criterion Value	Entry Score (end of AGAL-1-002-98 score)	Statistic	AGAL-005-99 6-month score			
			placebo/r-h α Gal n=21		r-h α Gal/r-h α Gal n=22	
			0	non-0	0	non-0
1%	0	n	0	0	8	0
	Non-0	n	17	4	11	3
10%	0	n	1	0	18	1
	Non-0	n	20	0	3	0

Note: Analysis done on subject data set available at time of initial BLA submission

Effect of age and ethnicity; distribution of change scores

Genzyme presents the data on age as dichotomized at the age of 30. The one subject who started the trial with a "0" score and was a "nonzero" at 6 months of the extension was over 30. The one subject who started the trial as a "0" and ended with a "non-0" was classified as "White." Since all placebo subjects and all but 1 r-h α Gal-treated subjects had a 6-month score of "0" score, the analysis of change scores primarily reflects the distribution of entry scores.

Endpoint: McGill short form pain questionnaire

Table 49 shows that there were no differences between the groups from entry into the trial to 6 months of AGAL-005-99. Baseline in the table is the baseline value in AGAL-1-002-98.

Table 49. AGAL-005-99: McGill short form results (means)

Pain measure	Time point	Placebo/ r-h α Gal n=29		r-h α Gal/ r-h α Gal n=29	
		n	mean value	n	mean value
Sensory	entry*	29	1.8	29	2.9
	change, baseline** to 6 months	25	-3.2	28	-2.6
	change, entry to 6 months	25	0.1	28	0.1
Affective	Entry	29	0.3	29	0.6
	change, baseline to 6 months	25	-1.3	28	-1.0
	change, entry to 6 months	25	0.0	28	0.2
Total	Entry	29	2.1	29	3.5
	change, baseline to 6 months	25	-4.5	28	-3.6
	change, entry to 6 months	25	0.1	28	0.4
Present pain intensity	Entry	29	0.6	27	0.7
	change, baseline to 6 months	25	-0.7	26	-0.8
	change, entry to 6 months	25	0.0	25	-0.2
Visual analog scale (VAS)	Entry	29	0.8	29	1.3
	change, baseline to 6 months	25	-1.7	28	-1.3
	change, entry to 6 months	25	-0.3	28	-0.2

*entry into AGAL-005-99

**baseline of AGAL-1-002-98

Endpoint: GL-3 in skin and heart

Table 50 shows summary statistics for skin and heart scores at baseline, visit 11, and 6 months post-entry.

Table 50. Skin and heart biopsy in AGAL-1-002-98 and AGAL-005-99 (mean \pm std. dev.)

Tissue	Treatment group	n	AGAL-1-002-98		AGAL-005-99
			Baseline	Visit 11	6 months post-entry
Skin	Placebo/r-h α Gal	n	29	29	26
			2.3 \pm 0.80	2.2 \pm 0.71	0.0 \pm 0.20
	R-h α Gal/r-h α Gal	n	29	29	27
			2.0 \pm 0.74	0.0 \pm 0.0	0.0 \pm 0.19
Heart	Placebo/r-h α Gal	n	29	29	18
			0.9 \pm 0.53	1.2 \pm 0.60	0.3 \pm 0.46
	R-h α Gal/r-h α Gal	n	29	29	22
			0.9 \pm 0.44	0.3 \pm 0.54	0.1 \pm 0.35

Table 51 shows the results expressed as 0 and non-0 status.

Table 51. Skin and heart biopsy results in AGAL-1-002-98 and AGAL-005-99

Tissue	Treatment group	AGAL-1-002-98				AGAL-005-99	
		Baseline		Visit 11		6 months post-entry	
		zero	non-zero	zero	non-zero	zero	non-zero
Skin	Placebo/r-h α Gal	2	27	1	28	25	1
Heart	R-h α Gal/r-h α Gal	2	27	29	0	26	1
	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

The great majority of placebo crossovers had a 6-month AGAL-005-99 score of 0.

Comment

The skin and heart data show a similar treatment effect to that seen in the kidney biopsies.

Endpoint: Urinary GL-3 levels

Upon request, Genzyme provided summary data regarding 24-hour urinary GL-3 levels during AGAL-005-99. Table 52 shows 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. The interpretation of these data is complicated by the fact that this is a subgroup analysis. The data themselves show a remarkable amount of variability, making quantitative discriminations between the groups difficult. Baseline data (baseline in AGAL-1-002-98) for these subjects was not provided.

Table 52. AGAL-005-99: Change in urinary GL-3 levels (nmol/filter) at 6 months

Statistic	Placebo/r-h α Gal n=22		r-h α Gal/r-h α Gal n=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

Plasma GL-3 levels

Although plasma GL-3 was not an endpoint, upon request, Genzyme provided plasma GL-3 results. Table 53 shows that subjects switched to active treatment in AGAL-005-99 experienced a decrease in plasma GL-3; those maintained on active treatment from AGAL-1-002-98 showed much smaller absolute changes, which is not unexpected since the entry values were significantly lower.

Table 53. AGAL-005-99: Plasma GL-3 levels (ng/ml) at entry and percent change at 6 months

Statistic	Placebo/r-h α Gal		r-h α Gal/r-h α Gal	
	entry	% change	entry	% change
n	28	28	28	28
Mean	14	-81	3.6	-21
Median	9.6	-100	0	0
Std. deviation	10.8	50.5	8.4	58.5
Min., Max.	0,39	-100,137	0,41	-100,166

Comments on plasma and urinary GL-3 observations

The reduction in plasma GL-3 observed with r-haGal 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-haGal.

Other endpoints

- Vibration detection threshold (U.S. subjects only). Values are reported for 11 subjects (U.S. only) in each treatment group at the 6-month point of the extension. End-of-trial scores from AGAL-1-002-98 were used for entry scores (for the placebo/r-h α Gal and r-h α Gal/r-h α Gal groups, 15.9 and 16.7, respectively); both groups experienced an increase toward more abnormal (for the placebo/r-h α Gal and r-h α Gal/r-h α Gal groups, 5.3 and 4.9, respectively). There were no notable differences in response between the two treatment groups.
- The Total Symptom Score. This was measured in 27 and 28 subjects in the placebo/r-h α Gal and r-h α Gal/r-h α Gal groups, respectively. No subscale score for either group was over 0.5 and the greatest absolute change from entry in a subscale score was 0.2 points. Genzyme reports that the changes observed in each group were "not clinically relevant." The total score difference (sum of 4 subscale scores) from baseline of AGAL-1-002-98 to the 6-month time point of AGAL-005-99 in subjects continued on active treatment was -1.2.

- SF-36 Health Status Survey, measured in almost all subjects (see Table 54). Scores on most subscales, with the exceptions of vitality, social functioning, and mental health, showed some improvements in both groups (general health score improved slightly in placebo/r-h α Gal subjects and deteriorated slightly in r-h α Gal/r-h α Gal-treated subjects). Genzyme does not comment on the clinical meaning of the changes seen; in addition, the overall interpretation of questionnaire data in an open-label trial is problematic due to the potential for subject bias.

Table 54. SF-36 scores at baseline of AGAL-1-002-98 and at entry and 6 months of AGAL-005-99*

Category of subscale		Placebo/r-h α Gal			r-h α Gal/r-h α Gal		
		Baseline (AGAL-1-002-98)	entry	6 months	Baseline (AGAL-1-002-98)	Entry	6 months
Physical functioning	<i>n</i>	29	29	25	29	29	28
		70.5 \pm 4.1	72.6 \pm 4.4	78.6 \pm 4.3	63.4 \pm 4.6	69.7 \pm 4.7	72.5 \pm 4.7
Role limitations due to physical	<i>n</i>	29	29	25	29	29	28
		43.1 \pm 7.4	53.4 \pm 7.9	70 \pm 7.6	39.7 \pm 6.9	63.2 \pm 7.6	60.7 \pm 7.8
Body pain	<i>n</i>	29	29	25	29	29	28
		49.8 \pm 4.3	63.9 \pm 4.3	71.3 \pm 4.8	52.8 \pm 4.2	61.9 \pm 3.3	64.9 \pm 3.9
General health	<i>n</i>	29	28	25	29	28	27
		53.7 \pm 2.4	54.2 \pm 2	55.8 \pm 2	53.1 \pm 2	54.1 \pm 1.6	52.2 \pm 1.7
Vitality	<i>n</i>	29	28	25	29	29	28
		56.4 \pm 1.8	58 \pm 1.6	53.2 \pm 1.7	57.6 \pm 2.3	58.1 \pm 1.9	56.1 \pm 2
Social functioning	<i>n</i>	29	29	25	29	29	28
		48.7 \pm 2.2	48.3 \pm 1.6	47.5 \pm 1.6	50.0 \pm 1.8	47.8 \pm 2.5	45.5 \pm 1.5
Role limitations due to emotional	<i>n</i>	29	29	25	29	29	28
		72.4 \pm 7.0	75.9 \pm 6.6	85.3 \pm 7	55.2 \pm 8.2	69 \pm 8.1	72.6 \pm 7.5
Mental health	<i>n</i>	29	28	25	29	29	28
		65 \pm 1.5	65.6 \pm 1.7	65.1 \pm 1.5	62.3 \pm 2.0	67.7 \pm 1.9	67 \pm 1.6
Physical component scale	<i>n</i>	29	28	25	29	28	27
		39.5 \pm 1.8	42.8 \pm 1.8	46.1 \pm 2	39.5 \pm 1.7	42.8 \pm 1.7	43 \pm 1.8
Mental component scale	<i>n</i>	29	28	25	29	28	27
		45.5 \pm 1.1	45.1 \pm 0.9	44 \pm 1.1	43.5 \pm 1.2	44.9 \pm 1.2	44.3 \pm 1

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

- Glomerular filtration rate, in all subjects: Table 55 is extracted from a 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. Genzyme's ANOVA on these data yielded a p-value of 0.524.

Table 55. GFR (mean \pm st. dev) in AGAL-1-002-98 and at 6 months of AGAL-005-99

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

CBER analyzed GFR as calculated by inulin technique for same-subject data sets (Table 56). There appears to be a baseline imbalance in GFR in this subset. Subjects who started on placebo had a small increase in GFR that continued after they were switched to r-h α Gal, while those who started on r-h α Gal showed an initial small increase followed by a decrease to baseline with continued

treatment. These data must be interpreted with caution, as they represent a nonrandomized subset of the data. Overall, no conclusion can be drawn reliably about the effect of r-h α Gal on GFR from these data, but these data do not provide evidence of any improvement with r-h α Gal treatment.

Table 56. GFR (mean \pm std. error) by inulin technique only in AGAL-1-002-98 and at 6 months of AGAL-005-99 *

Trial	Visit	Treatment group*	
AGAL-1-002-98		Placebo n=19	r-h α Gal n=17
	Baseline	96 \pm 7	75 \pm 6
	Visit 11	101 \pm 8	94 \pm 9
AGAL-1-002-98 and AGAL-005-99		Placebo n=17	r-h α Gal n=15
	Baseline	96 \pm 8	78 \pm 6
	Visit 11	102 \pm 9	93 \pm 9
	6-month	116 \pm 6	79 \pm 8

*as-treated group, considered the same as intent-to-treat

- Autonomic status as described in the review of AGAL-1-002-98, assessed in 11 subjects in the U.S. 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. QSART mean values at entry to AGAL-005-99 were abnormal for both groups at 42.1 and 55.9 (units not provided) for the placebo/r-h α Gal and r-h α Gal/r-h α Gal groups, respectively, with decreases for both (20 and 48, respectively).
- Serum creatinine, measured in all subjects. While there is arguably a small increase in serum creatinine among placebo subjects switched to r-h α Gal, there is virtually no change in subjects continued on r-h α Gal for 11 months. The results depicted in Table 57 show no clinically notable effect of r-h α Gal on serum creatinine.

Table 57. Serum creatinine (mg/dl, mean \pm std error)

Trial	Visit	Treatment group	
AGAL-1-002-98		Placebo n=29	r-h α Gal n=29
	Baseline	0.79 \pm 0.04	0.83 \pm 0.04
	Visit 11	0.79 \pm 0.05	0.89 \pm 0.04
AGAL-005-99		Placebo n=27	r-h α Gal n=28
	6-month	0.91 \pm 0.11	0.89 \pm 0.04

- 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 collected only at the 6-month time point, 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). This observation merits continued surveillance, as increases in protein excretion may signal increased renal dysfunction.

Summary comments on efficacy

Histological analyses showed that reductions of substrate from capillary endothelium of kidney, skin, and heart were sustained in subjects continuing to take 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.

Clinical endpoints, as well as measures of renal function, continued to show no effect of treatment after 6 months of AGAL-005-99, representing a total of 11 months of treatment for r-h α Gal continuers.

Data on additional cell types and data on skin cell histology (including longer time periods of treatment) are reviewed in a separate document.

Longer-term data on renal function and clinical endpoints are reviewed in a separate document.

RESULTS: SAFETY

Note: Safety data from later time points in AGAL-005-99 are reviewed in a separate document.

Adverse events occurring between AGAL-1-002-98 and AGAL-005-99

Numbers of subjects with events whose start dates occurred between (but not including) the last infusion of AGAL-1-002-98 and the first infusion of AGAL-005-99 were small. Only 2 preferred terms occurred for 3 subjects or more in either group: heart valve disorders were reported in 3 subjects in the placebo/r-h α Gal group, and 5 in the r-h α Gal/r-h α Gal group, and a retinal disorder was reported for 3 subjects in the placebo/r-h α Gal group and 2 subjects in the r-h α Gal/r-h α Gal group. Other events occurred sporadically.

Deaths

There were no deaths in AGAL-005-99 to the cutoff date, August 31, 2000.

Adverse events

Serious adverse events

Table 58 shows the serious adverse events that occurred in AGAL-005-99 through August 31, 2000.

Table 58. AGAL-005-99: Serious adverse events through August 31, 2000

Treatment group	Subject	Days from entry into extension	Verbatim terms	Severity
r-h α Gal/r-h α Gal	109	196	Hematuria*	Severe
		197	Hematoma*	Mild
	115	56	Attempted Suicide	Severe
	202	197	Decreased blood pressure*	Moderate
		197	Hemorrhage*	Moderate
	605	238	Pericardial effusion*	Severe
		238	Pericardial rub*	Severe
		238	Pericarditis*	Severe
804	80	Basal cell carcinoma (cheek)	Moderate	
placebo/r-h α Gal	201	32	Tachycardia**	Moderate
		32	Hypertension"	Moderate
	304	98	Pruritic Urticaria**	Mild
	306	55	Tightness in chest**	Severe
		55	Tightness in throat**	Severe
	502	53	Dizziness	Moderate
		53	Worsening angina	Mild
	506	242	Bradycardia	Severe
		242	Cardiac output decreased	Severe
	603	36	Chest pain	Mild
	703	72	Vertigo	Mild
		79	Hypoacusia	Severe
	708	-12	Pericardial effusion* ¹	Mild
		9	Thoracic pain with oppression*	Moderate
		175	Transitory ischemia (hand)	Moderate
	805	66	Loss of visual acuity	Moderate
		66	Macular edema	Moderate
128		Fever**	Severe	
128		Shivering**	Severe	
	128	Very intense tachycardia**	Severe	

*related to biopsy

**related to infusion of r-h α Gal¹ same observation had been noted after cardiac biopsy in AGAL-1-002-98

Most events were related to either a biopsy or infusion of product. Other events were not reported in close relation to an infusion.

The following are brief notes on the serious adverse events that were not reported in relation to a biopsy or infusion:

R-h α Gal/r-h α Gal group

- Subject 804 is a 43 year-old; the carcinoma was excised.
- Subject 115 was hospitalized for 2 days after a suicide attempt, which was reportedly due to frustration with his Fabry's disease. No prior history of depression or suicide attempt is reported.

Placebo/r-h α Gal group

- Subject 502 had a history of angina, dyspnea, and atrial fibrillation. Treatment was a check of his pacemaker.
- Subject 506 had a history of myocardial infarction and heart failure. He required a pacemaker insertion.
- Subject 603 was a 20 year-old with no reported cardiac history who developed chest pains; no objective tests were reported in the BLA; the only reported treatment was aspirin.
- Subject 703 developed decreased hearing and vertigo of unexplained causes; the hypacusis resolved but the vertigo was ongoing as of the writing of the safety report.

This subject had “a history of vestibulocochlear disorders, including a 10-year history of tinnitus, bilateral hearing loss since 1994, and vertigo in 1998 which persisted for several months.”

- Subject 708 developed hand pain and inability to use his hand of 2 hours' duration. Physical exam and echo Doppler did not show abnormalities. Treatment is not reported.
- Subject 805 experienced a sudden loss of vision in the right eye 10 days after infusion 5 with a loss of visual acuity from 20/20 to 5/10. Fluorescein angiography showed white spots on the macula, leading to a diagnosis of retinal white dots syndrome. It also showed mild vascular irregularities. Further monitoring showed “On 21 November 2000, visual acuity was recorded as 20/20 in both eyes. Funduscopy examination was normal.”

Comments regarding selected serious adverse events

Regarding tinnitus, Genzyme states that three subjects were using medications that have tinnitus as a side-effect (carbamazepine, gabapentin). One subject has a history of “ear whistle’ or mild buzzing in the ears.” The treatment assignments of the subjects are not presented. Because tinnitus occurs as a symptom in Fabry's disease, these cases do not support a safety concern.

Regarding arthrosis, Genzyme states that there was no contributory medical history in subjects with arthrosis, and there was no relationship of arthrosis to the administration of concomitant medications. Reports included “stiff neck,” “stiff hands/feet,” “stiff joints,” and joint stiffness and pain.” A literature search summary provided by Genzyme states that “descriptions of localized joint pain are fairly uncommon.” However, there is an arthropathy, primarily of the hands, associated with Fabry's disease.

Regarding vertigo and hypoacusia, a literature summary reports that the incidence of these conditions is unknown, but that they are reported in Fabry's disease.

Regarding visual acuity loss, no more clinical details are provided. A literature summary reports that visual acuity loss is “rare.” There are reports of white spots on the retina, and the subject in question had “white dots syndrome.”

Comment

The group switched to r-haGal experienced several serious cardiac adverse events, but serious adverse events of this nature did not occur in the r-haGal-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.

Infusion reactions

The majority of subjects experienced infusion reactions. Table 59 shows the numbers of subjects with any adverse event in groups of infusion-related syndromes.

Table 59. AGAL-005-99: Subjects with infusion-related adverse events

Adverse Events	Subjects with any infusion-related event out of the total trial population of 58 <i>n=34</i>
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

Genzyme states, “Treatment has consisted of a reduction in infusion rate, with various combinations of antihistamines, inhaled beta agonists, NSAIDs, or steroids.” All subjects other than subject 304 (withdrawn for a (+) skin test) have continued taking r-h α Gal.

Sites were to notify Genzyme Pharmacovigilance for serum testing in the event of a moderate to severe hypersensitivity reaction. Table 60 shows laboratory data and infusion number for subjects with infusion reactions that were reported to Genzyme Pharmacovigilance. It shows that more of these events occurred in subjects crossed over to r-h α Gal treatment from placebo during AGAL-005-99, and that the majority of subjects (15/25) were crossover subjects. 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. 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 60. AGAL-005-99: Anti- α -gal IgG and IgE and complement activation in infusion reactions reported to Genzyme Pharmacovigilance

Treatment	Subject Number	Infusion Number	IgE Test Result	IgG Test Result	Complement Activation
Placebo/ R-h α Gal	0105	9	-	+	-
		0110	7	-	+
	0116	13	Not tested	+	Not tested
		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	-	+	+
	0115	7	Not tested	+	Not tested
		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	-	+	+
		11	Not tested	+	Not tested
	0604	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 (-).

Comment

These results suggest that infusion reactions to r-haGal decrease in incidence with time, but that they continued in treated subjects for almost a year. Although clinical details of the infusion events are not provided, it can be inferred from the protocol that moderate to severe reactions (those necessitating IgE testing) occurred out to almost a year of treatment in some subjects (infusion 9 and 12 in two r-haGal-treated subjects). 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.

Adverse events leading to withdrawal

One subject was withdrawn during the 6-month period of this report. Subject 304 experienced hypersensitivity symptoms during an infusion. He was withdrawn when he tested (+) on a skin test.

Nonserious adverse events

Table 61 shows subjects with adverse events, organized by severity, that occurred from the time of the first infusion in AGAL-005-99 to up to the 6-month interim analysis time point, in at least 3 subjects per treatment group. This table, created by CBER, differs somewhat from the table provided in the BLA, which erroneously contained some events that occurred in AGAL-1-002-98. In addition, Table 61 omits 11 adverse events in which the start date was listed as unknown; these were miscellaneous events occurring in both groups.

Table 61. AGAL-005-99: Numbers of subjects with adverse events on or after the first day of infusion, occurring in 3 or more subjects per treatment group, by severity

Preferred term	Placebo/r-h α Gal n=29				r-h α Gal/r-h α Gal n=29			
	Mild	Moder.	Severe	Total	Mild	Moder.	Severe	Total
Rigors	11	3	1	15	8	3	2	13
Rhinitis	7	4	0	11	7	0	0	7
Temperature changed sensation	10	1	0	11	8	2	0	10
Fever	9	0	1	10	4	1	1	6
Pain	5	2	2	9	4	0	0	4
Chest pain	5	2	1	8	2	0	0	2
Albuminuria	5	2	0	7	5	2	0	7
Edema dependent	6	0	0	6	3	2	0	5
Tremor	4	2	0	6	2	1	0	3
Upper respiratory tract infection	6	0	0	6	3	0	0	3
Vomiting	4	2	0	6	4	0	1	5
Dyspnea	4	1	0	5	1	2	0	3
Flushing	3	2	0	5	1	0	0	1
Headache	5	0	0	5	11	2	0	13
Influenza-like symptoms	4	1	0	5	0	5	0	5
Nausea	3	2	0	5	6	0	0	6
Pharyngitis	5	0	0	5	4	0	0	4
Bronchospasm	4	0	0	4	0	1	0	1
Diarrhea	4	0	0	4	2	0	1	3
Fabry pain	1	3	0	4	2	1	1	4
Post-operative pain	2	2	0	4	4	0	0	4
Pruritus	4	0	0	4	1	0	0	1
Somnolence	4	0	0	4	1	0	0	1
Abdominal pain	3	0	0	3	3	0	0	3
Back pain	1	1	1	3	0	2	1	3
Cardiomegaly	3	0	0	3	3	1	0	4
Fatigue	2	1	0	3	1	0	0	1
Leg pain	3	0	0	3	1	0	1	2
Palpitation	3	0	0	3	0	1	0	1
Paraesthesia	2	0	1	3	4	0	1	5
Renal function abnormal	2	1	0	3	2	1	0	3
Syncope	3	0	0	3	1	1	0	2
Bronchitis	1	1	0	2	2	1	0	3
Dizziness	1	1	0	2	3	0	0	3
Hypertension	1	1	0	2	2	1	0	3
Myalgia	2	0	0	2	1	2	0	3
Asthenia	0	1	0	1	2	1	2	5
ECG abnormal	1	0	0	1	3	0	0	3
Coughing	0	0	0	0	3	0	0	3

There were few “severe” adverse events in either group.

The placebo/r-h α Gal group experienced a similar total number of infusion-related events as was experienced in the r-h α Gal-treated group from AGAL-1-002-98. The incidence of post-operative pain was dramatically lower in AGAL-005-99 in both groups, probably in part because

there were ½ as many biopsies counted for this report period. The following adverse events were examined in more detail:

- Chest pain: Chest pain not occurring on the day of infusion occurred in 2 subjects in either group; 4 of the 6 remaining subjects in the placebo/r-h α Gal group had chest pain on the day of infusion that was deemed at least possibly related to the infusion of r-h α Gal.
- The numbers of headaches in the r-h α Gal/r-h α Gal group exceeded that in the placebo/r-h α Gal group, but was equal to the number that the same group experienced in AGAL-1-002-98. The neurologically-related adverse events paresthesia and dizziness were not similarly increased. The etiology of the difference between the groups is unclear, but does not seem related to increased time of exposure to r-h α Gal.
- Pain: Two placebo and no r-h α Gal-treated subjects experienced an adverse event categorized as “pain” on the day of infusion. The great majority of adverse events descriptions are consistent with pain due to Fabry's disease.
- Bronchospasm: The discrepancy in the numbers of subjects with bronchospasm is accounted for by the difference between treatment groups in the numbers with bronchospasm on the day of infusion, except for 1 placebo/r-h α Gal subject with an episode of mild wheezing not on the day of infusion.
- Asthenia: All cases, except in one r-h α Gal-treated subject, were noted as having recovered. Adverse event descriptions are not detailed enough to provide additional insight into these events.
- ECG abnormalities: Most of these events were nonspecific ST wave abnormalities. There was no remarkable increase in the incidence of clinical cardiac adverse events, so the small discrepancy does not appear to be clinically significant.
- Hypertension was reported primarily as an infusion-associated event, and its low incidence in the group switched from placebo was similar to that in the r-h α Gal group from AGAL-1-002-98.
- Myalgia (as a sentinel symptom of serum sickness) occurred at a low incidence in both groups, not increased in the group with the longer duration of exposure (r-h α Gal/r-h α Gal). The data listings were searched for preferred terms with “arth,” as a means to detect arthritides possibly associated with serum sickness. One subject, in the continued r-h α Gal group, had an event categorized as “arthrosis,” and described as “rigid joints” in association with an infusion. These events do not suggest the development of clinical serum sickness with 11 months’ treatment with r-h α Gal.

Comments

The data do not suggest that there is an increase in toxicity with continued exposure to r-haGal. Further, they show a general agreement of the overall adverse event profile of the subjects switched from placebo to r-haGal in AGAL-005-99 and those exposed to r-haGal for the first time in AGAL-1-002-98.

Concomitant pain medication

There were no notable intertreatment group differences in the use of medications used for pain.

Laboratory data

CBER reviewed laboratory abnormalities listed as adverse events, mean changes in hematology parameters and routine serum chemistries from entry to AGAL-005-99 to the 6-month time point, and numbers of subjects who started the trial with normal values and ended with abnormal ones. This review did not reveal a toxicity of r-h α Gal. As can be seen in Table 61, albuminuria was the laboratory adverse event that occurred in the greatest number of subjects, and this was equally distributed between the treatment arms.

Antibody

Only 1/25 seropositive subjects became seronegative during AGAL-005-99 (subject 109, who became persistently seronegative at the first visit of AGAL-005-99). Another subject became seronegative at the last visit in AGAL-005-99 for this report. Subject 116, treated with placebo in AGAL-1-002-98, seroconverted during that period and remained seropositive at least to the end of the period of observation of AGAL-005-99.

In the placebo/r-h α Gal group 25/29 subjects seroconverted after enrollment in AGAL-005-99 and prior to infusion 15, or approximately 6 months (Table 62). Two of the 5 subjects treated with r-h α Gal who were seronegative during AGAL-1-002-98 seroconverted during AGAL-005-99.

Table 62. Trial AGAL -005-99: Numbers of subjects by visit at which anti- α galactosidase IgG was first observed

Trial visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Placebo/r-h α Gal	0	1	8	3	4	2	4	1	0	0	0	0	0	2	25
R-h α Gal/r-h α Gal	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2

Table 63 shows the titer change at the last visit for this safety report, among subjects who seroconverted during AGAL-1-002-98 and AGAL-005-99. Most subjects remained near their original titer.

Table 63. Titer at end of observation period of AGAL -005-99 relative to 1st titer, among subjects who seroconverted in trial indicated

Titer	Seroconverted in AGAL-1-002-98 <i>n</i> =24*	Seroconverted in AGAL-005-99 <i>n</i> =27
within 1 dilution	14	16
less by ≥ 2 dilutions	8**	3
more by ≥ 2 dilutions	2**	8

*omits placebo subject

**includes 1 subject whose last titer was not at the last visit of observation in AGAL-005-99

Comments

Nearly everyone exposed to r-haGal generated an IgG antibody response to it. When correlated with the histology results, it can be inferred that the presence of antibodies did not result in a reaccumulation of substrate.

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 capillary endothelium of the kidney, heart, and skin. It is noteworthy that histologically-assessed outcomes were stable during continued treatment with r-h α Gal, despite the nearly universal seroconversion of subjects in AGAL-1-002-98. In addition, placebo subjects crossed over to active treatment, most of whom became seropositive, showed similar reductions in substrate as their active-treated counterparts in AGAL-1-002-98. However, there were no additional clinical findings or findings from other laboratory evaluations that would reveal a clinical benefit or extend the histological finding of kidney capillary endothelial substrate reduction.

There were no additional safety concerns among those who received an additional 6 months of treatment with r-h α Gal. However, the concern over the occurrence of infusion reactions in both crossover and continued treatment subjects remains.

120-DAY SAFETY UPDATE

CONTENTS OF THE REPORT

The 120-day safety report contains the safety and antibody data already presented in the review of AGAL-005-99. In addition, it contains serious adverse event data from all other ongoing trials up to August 31, 2000. These trials include:

- Open label extension to FB9702-01; AGAL-006-99, in which subjects receive r-h α Gal at approximately 1 mg/kg every 2 weeks
- Open label trial, AGAL-007-99 in Japan, in which subjects receive r-h α Gal at approximately 1 mg/kg every 2 weeks
- Single-subject trial, with administration of r-h α Gal projected to be every 2 weeks in a subject with end-stage renal disease

RESULTS

Deaths

One death occurred during the time period covered by this report. The subject with end-stage Fabry's disease died of sepsis due to an infected peritoneal dialysis catheter shortly after receiving his only dose of r-h α Gal.

Serious adverse events

In trial AGAL-006-99 a 39 year-old man with a history of mitral valve repair and pacemaker inserted for an unspecified arrhythmia experienced axillary and arm pain. Diagnostic tests included ECG, thallium stress test, and cardiac catheterization; these did not reveal a proximal cause for pain. The subject recovered without sequelae.

No serious adverse events have been reported from the Japanese trial.

Comments

The reported events do no point to a significant safety concern not seen in the review of clinical trials fb97, AGAL-1-002-98, and AGAL-005-99.

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; anti-r-h α Gal IgE has been detected. Genzyme knows of no predisposing factors to the development of infusion reactions. Although no subject discontinued from treatment due to infusion reactions, the rarity of other treatments and the desire to contribute to the development of clinical data may have been a consideration for some subjects.

Hypertension as an adverse event occurred in the great majority of subjects in the FB9702-01, but was not severe. Hypertension did not occur to a great extent in AGAL-1-002-98 or its open-label extension, AGAL-005-99. The reasons for this are not clear, but may relate to reporting differences or to differences, not elucidated, in lots of product used in the trials.

Although the great majority of subjects developed IgG antibody to r-h α Gal, there was no evidence of immune complex disease. This condition is difficult to diagnose from clinical symptoms, however, and preexisting renal disease in Fabry's disease, including albuminuria, could conceivably complicate the discovery of renal dysfunction due to immune complex disease.

POST-MARKETING EXPERIENCE

As of the submission of this BLA, the product had not been marketed anywhere. The BLA does not contain any postmarketing information.

FINANCIAL DISCLOSURE

Genzyme submits that, for the principal investigator and a list of selected subinvestigators for each site for AGAL-1-002-98:

- Genzyme had not entered into any financial arrangement whereby the value of compensation could be affected by the outcome of the trial (21 CFR 54.2(a));
- The clinical investigators required to disclose to Genzyme whether the investigator had a proprietary interest in the product or a significant equity in Genzyme as defined in 21 CFR 54.2(b) did not disclose any such interests; and
- No listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f).

Genzyme reports financial arrangements with two additional subinvestigators:

- 1) 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. Genzyme reports that the patents are assigned to Mt. Sinai, which has the right to grant licenses to the patents, and which has an arrangement to receive royalties from Genzyme in relation to these patents. Genzyme reports arrangements to pay Dr. Desnick a total of \$----- in 1999-2000 as a consulting fee for Fabry's disease, and \$----- as a consulting fee for ----- disease from 1999 to the first quarter of 2001. Genzyme has arrangements to provide grant money and scientist support to Mt. Sinai, as well. In summary, there is an indirect financial relationship between Genzyme and Dr. Desnick with regards to the outcome of AGAL-1-002-98.
- 2) Genzyme reports arrangements to pay Dr. Hans Aerts, a subinvestigator at the Academisch Medisch Centrum, a total of \$----- for 1999-2000 in the form of a research grant.

For these subinvestigators, Genzyme states that

- There are no financial arrangements where the value of compensation for conducting the study could be influenced by the outcome of the study.
- There is no equity interest in Genzyme.
- Dr. Desnick's involvement was "largely limited to activities including referring potential study patients for screening, and obtaining informed consent from the study participants. As a sub-investigator, Dr. Desnick provided medical coverage on rare occasions in the absence of the principal investigator."
- Dr. Aerts had "no involvement with the conduct of the study although he was listed as a subinvestigator."

Inspection by CBER did not point to a concern over the conduct of AGAL-1-002-98 at Mt. Sinai.

In summary, the financial arrangements noted by Genzyme, and the results of inspection of the Mt. Sinai site, do not cast doubt on the conclusions of the effect of r-h α Gal in this BLA.

CONCLUSIONS

The clinical data presented provide for the following conclusions:

- Intravenous administration of Genzyme's r-h α Gal results in reduction of capillary endothelial accumulation of the enzyme's substrate in various organs, including 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 latter conclusion is based upon a consideration of the small data base in the uncontrolled trial FB9702-01 and on comparison of actively treated subjects to placebo in bulk residual substrate levels in kidney and other organs from AGAL-1-002-98. 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 is reasonable to judge that the product could show clinical benefit.
- The clinical trials failed to show clinical benefit on a wide range of tests of neurologic, renal, and cardiac function. This finding weakens confidence in the clinical importance of the reduction of kidney interstitial capillary endothelial cell GL-3 levels that constituted the primary endpoint of pivotal trial AGAL-1-002-98. Whether a longer trial could have demonstrated a benefit is an open question.
- Infusion reactions were common and significant, sometimes occurring despite premedication, and requiring infusion rate adjustments and medical therapies. As of the submission of the BLA, 1 subject had been withdrawn due to the development of a skin reaction indicating IgE antibody formation. These reactions are a significant concern.
- Noninfusion-related toxicities appeared to be minimal.
- Antibody formation was extremely common, and persistent. The continued presence of antibodies poses a potential safety risk for the development of continued infusion reactions. In addition, there is 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.

RECOMMENDATIONS

A number of issues remained after review of this original submission to the BLA, and additional data were requested. The main issues were:

- 1) The generalizability of the histological effects to other cell types, including those in the kidney
- 2) The potential loss of the histological effects over time, especially given the widespread development of antibody to the product
- 3) The pattern of toxicities over time, especially infusion reactions, given the data on development of antibody.

Data relevant to these issues are reviewed in another document.

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