

Fabrazyme[®]

(agalsidase beta)

Briefing Document

Available for public disclosure without redaction

Prepared by Genzyme Corporation for the
Endocrinologic and Metabolic Drugs Advisory Committee
Center for Drug Evaluation and Research (CDER) Meeting
13 January 2003

EXECUTIVE SUMMARY

Fabry Disease and Unmet Medical Need

Fabrazyme is indicated for use as long-term enzyme replacement in patients with Fabry Disease. Fabrazyme treats the underlying pathology of Fabry Disease by significantly clearing neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3) to normal or near normal levels from vascular endothelium of kidney, heart and skin.

Fabry disease is a very rare, monogenic, X-linked, hereditary disorder resulting from mutations in the gene encoding the lysosomal hydrolase, α -galactosidase A. This leads to the progressive intracellular lysosomal accumulation of several neutral glycosphingolipids, most prominently, globotriaosylceramide (GL-3), in many cell types throughout the body. Although multiple cell types accumulate GL-3, it is the involvement of the capillary endothelial cells that leads to the devastating renal, cardiac, and cerebrovascular clinical manifestations of disease.

The clinical course of Fabry disease is indolent, yet relentlessly progressive over many years. While there is much heterogeneity in the clinical course, patients with classic Fabry disease usually present in childhood with a complex pain syndrome consisting of both acute and chronic pain. Patients have episodic acroparesthesias and severe pain crises, associated with physical exertion, stress, and warm temperatures. Patients also frequently have hypohidrosis and gastrointestinal symptoms. While the pain syndrome often wanes over time, significant morbidity and mortality results from renal insufficiency that usually begins in the fourth decade of life, resulting in patients requiring dialysis or a renal transplant. Most often, later in life cerebrovascular and cardiovascular involvement lead to strokes and myocardial infarctions.

Fabry patients, in much the same way as patients with hypercholesterolemia, can frequently be asymptomatic for many years while abnormal accumulations of GL-3 occur in the vasculature. However, only after years of pathological deposition and accumulated damage, does clinically evident end organ failure occur. Once the end organ failure has manifested itself, it is often irreversible. Any truly effective intervention should be aimed at preventing the end organ damage from occurring. Intervention is less effective in reversing the disease once fibrosis or infarction has occurred. Current therapy for Fabry patients consists merely of palliation of disease symptoms. The patient population in the United States is approximately 3500 males.

(For more detail, refer to Sections 1.2, 1.3, and 1.4.)

Treatment of Fabry Disease with Fabrazyme®

Genzyme has developed Fabrazyme® containing the active ingredient recombinant human α -galactosidase A (r-h α GAL, agalsidase beta) as enzyme replacement therapy for patients with Fabry disease. Recombinant-human α GAL is a highly purified recombinant form of the naturally

occurring human glycoprotein and is produced in Chinese hamster ovary cells transfected with a DHFR-vector carrying the cloned cDNA of the human α GAL-A gene.

Fabrazyme is taken up by cells by the mannose-6-phosphate receptor, is transported to the lysosome and possesses enzymatic activity as demonstrated in various in vitro and in vivo preclinical and clinical studies. Hence, replacement therapy with Fabrazyme restores the missing enzyme activity and leads to the clearance of neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3) in the lysosomes of various cells that would, in Fabry disease, show pathologic accumulation. Therefore, it would be expected that prolonged treatment with Fabrazyme resulting in removal of GL-3 accumulation will lead to normalization or possible improvement in organ function.

(For more detail, refer to Section 1.6.)

Regulatory Status of Fabrazyme

Fabrazyme has been granted Orphan and Fast Track designation by FDA. The BLA was submitted under the Accelerated Approval mechanism described in 21 CFR Part 601, Subpart E in June 2000 and was given Priority Review status because of the serious and life-threatening nature of Fabry disease and the potential therapeutic benefit that Fabrazyme may provide over current palliative therapies. The accelerated approval mechanism was developed to allow expedited marketing based on clinical evidence of safety and efficacy using a mutually agreed upon surrogate endpoint reasonably likely to predict clinical benefit. This process is used in the case of serious or life-threatening diseases where the proposed product provides meaningful therapeutic benefit over existing treatments. This mechanism requires the sponsor to verify and describe clinical benefit through a post-marketing study.

Fabrazyme has been approved for marketing in over 25 countries, including the European Union and Australasia.

(For more detail, refer to Section 1.7.)

Fabrazyme Clinical Development Plan

1.) Fabrazyme Dose and Dosing Interval

Fabrazyme has been studied in several non-clinical pharmacokinetic, pharmacodynamic, and toxicology studies in several models and species, including the α GAL Knock-out SV129 mice. Based upon these studies, as well as the dose-ranging (0.3, 1.0, and 3.0 mg/kg), pharmacokinetic, pharmacodynamic, and safety results obtained in a 15 patient Phase 1/2 study, a dose of 1.0

mg/kg administered intravenously every two weeks was chosen. This dosing regimen offered the optimal balance between GL-3 clearance and the frequency of infusion associated reactions.

(For more detail, refer to Section 3.2.1.)

2.) Rationale for Primary Endpoint Selection: GL-3 Clearance

In designing a Phase 3 Placebo-Controlled Study to evaluate the therapeutic benefit of Fabrazyme for treatment of Fabry disease, careful consideration was given to the major clinical manifestations of the disease.

Renal insufficiency has been the major cause of morbidity and mortality in Fabry patients. However, based on the pathophysiology of the renal disease and the irreversibility of glomerulosclerosis, it was concluded that the most appropriate clinical endpoint would be to focus on preserving renal function, thereby avoiding the most devastating common complication of the disease. The likelihood that one could improve renal function once glomerulosclerosis had occurred was felt to be unlikely.

Since renal insufficiency progresses over the course of years, it was determined that to properly design a study using preservation of renal function as the primary endpoint, particularly among patients with relatively normal renal function, would require a relatively large number of patients studied over several years, as in studies of patients with diabetes. This was not felt to be feasible in an ultra-orphan population.

Careful consideration was also given to designing a study focusing solely on the subset of Fabry patients who already have abnormal renal function based on increased serum creatinine and decreased glomerular filtration rate (GFR). However, our analysis revealed that even focusing on this narrow subset of Fabry patients would still require a study of approximately three years' duration. Such a study is an important part of our Phase 4 program.

While **pain** is often a presenting symptom of the disease and improvement in pain was ultimately incorporated as a secondary endpoint, it was not chosen as the primary endpoint for several reasons. (See Section 3.2.2.1) Primarily, pain is subjective in nature and highly variable in extent, making it challenging to measure quantitatively. In addition, Fabry pain often wanes over time and it would be very difficult, if not impossible, to account for this in the sizing of such a study in this ultra-orphan disease population. It was calculated that to study pain improvement as the primary endpoint required statistically powering the study with more than 100 Fabry patients per group who had significant pain on pain medications in consideration of the placebo effect that is often observed in a study setting and based upon other well-controlled pain studies for other indications. This was not feasible given the very small size of this patient population.

Prevention of clinically significant **cardiac** or **cerebrovascular** events were also considered as primary endpoints. However, these events are episodic in nature with an event rate so poorly documented that determination of sample size and study duration were not feasible. Additionally, they are confounded by common concomitant conditions such as hypercholesterolemia and hypertension.

3.) Selection of Primary Surrogate Endpoint for Pivotal Phase 3 Placebo-Controlled Clinical Study

Because of the feasibility concerns outlined above surrounding the conduct of a properly powered pivotal study using a clinical endpoint in this ultra-orphan disease, and after extensive discussions between Genzyme, FDA and experts in the field, a surrogate endpoint likely to predict clinical benefit was mutually identified and agreed upon with FDA as being appropriate for studying as the primary endpoint in the Phase 3 Placebo-Controlled Study.

The mutually agreed upon primary endpoint was the histologic clearance to normal or near normal levels (i.e., 0 score on a scale of 0-3) of GL-3 accumulations from the interstitial (peritubular) capillary endothelial cells of the kidney. (For more detail, refer to Section 3.2.2.1.)

To summarize, the rationale for agreeing to this primary endpoint was that:

- renal failure is the most common devastating feature of Fabry disease;
- glomerulosclerosis present in Fabry disease is primarily a result of vascular damage;
- while multiple cell types accumulate glycosphingolipids (i.e. GL-3), the clinical-pathological correlation in two milder variant Fabry patient populations strongly support the concept that endothelial cell deposition leads to the ultimate end organ damage and subsequent clinical deterioration; Both female heterozygotes and cardiac variant patients generally have higher residual endogenous α GAL activity, very little GL-3 accumulation in endothelial cells (though extensive accumulation in cardiomyocytes and renal epithelial cells), and have a much milder clinical course of disease;
- this endpoint could be measured in a reasonable timeframe;
- statistical power calculations based on this endpoint showed that the study population required was appropriate for this rare disease; and
- clearance of GL-3 to normal or near-normal levels was felt to be clinically important and would be expected to predict normalization/stabilization of function and clinical benefit.
- for insidious diseases, such as kidney failure, that lack overt clinical symptoms until they are beyond the reach of medical therapy, histologic diagnosis often can be the only objective indicator for staging disease severity and gauging the success of therapy.

4.) Selection of Additional Endpoints

Secondary endpoints included a histologic assessment of reduction of GL-3 in endothelial cells of the heart and skin, changes in pain as assessed by the Short Form McGill Pain Questionnaire, and a biochemical assessment of GL-3 clearance from urine sediment and kidney tissue.

Numerous tertiary and other endpoints were investigated during the study including changes in plasma GL-3, renal function, and quality of life as assessed by the Short Form (SF)-36. In response to FDA questions, clearance of GL-3 in multiple renal cell types was also evaluated.

(For more detail, refer to Section 3.2.2.2.)

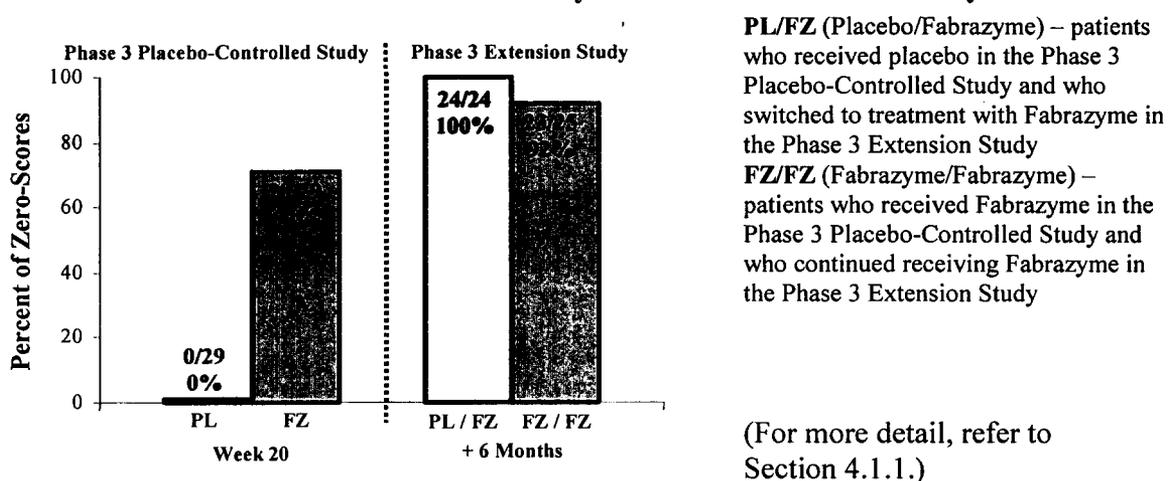
Summary of Efficacy

A Phase 3, double blind, randomized, placebo-controlled, multicenter study was conducted at 8 sites in 4 countries on 58 patients with Fabry disease. Patients received 1.0 mg/kg of Fabrazyme or placebo intravenously every 2 weeks, for a total of 11 infusions (20 weeks). Patients who successfully completed the Phase 3 Placebo-Controlled Study were eligible for entry into a Phase 3 Extension Study. All 58 patients completed the Phase 3 Placebo-Controlled Study and elected to enroll in the Phase 3 Extension Study. During this ongoing extension study, all patients are receiving Fabrazyme, and long-term safety and efficacy parameters are being monitored. This study will continue assessments for an additional 4.5 years, for a total of up to five years of patient treatment with Fabrazyme. Currently, this study is being conducted at 19 investigational sites in the U.S. and Europe.

1.) Primary Endpoint: Renal Interstitial Capillary Endothelial Cells

The results of the Phase 3 Placebo-Controlled Study and the Phase 3 Extension Study demonstrate that the single, prospectively agreed upon primary endpoint was reached with a high degree of statistical significance ($p < 0.0001$). 20/29 (69%) patients treated with Fabrazyme achieved a zero score in the renal capillary endothelium compared to 0/29 of the patients treated with placebo. This was consistent among the three, blinded renal pathologists, between different clinical sites, and among all patient subsets. This improvement was shown to be maintained by analyses of repeat kidney biopsies obtained at 6 months into the Phase 3 Extension Study and was confirmed in the patients that crossed over from placebo to Fabrazyme treatment. (Figure 1)

Figure 1 Kidney Histology: Response of Interstitial Capillary Endothelial Cells in the Phase 3 Placebo-Controlled Study and Phase 3 Extension Study



2.) Histologic Assessment of Additional Cell-Types of the Kidney

In response to questions from FDA, additional analyses on multiple additional cell-types were subsequently conducted to verify that clearance of GL-3 accumulation was not an isolated finding limited to interstitial capillary endothelial cells. These additional cell-types were retrospectively evaluated in the pathology slides obtained at Baseline and Week 20 of the Phase 3 Placebo-Controlled Study and 6 Months into the Phase 3 Extension Study. The additional kidney cell-types for analysis included glomerular endothelial cells, non-capillary (arteriolar) interstitial smooth muscle cells, non-capillary (arteriolar) interstitial endothelial cells, podocytes, distal convoluted tubules/collecting ducts, mesangial cells, and interstitial cells. At the end of the Phase 3 Placebo-Controlled Study, there was a highly statistically significant difference ($p < 0.001$) between the Fabrazyme and placebo treatment groups in the number of patients achieving a zero-score in several additional cell types: mesangial cells, glomerular capillary endothelium, interstitial cells and non-capillary endothelium. Nearly all of the patients with available data who had originally received placebo in the Placebo-Controlled study showed clearance to normal or near-normal levels of GL-3 accumulation in these additional kidney cell-types after 6 months of open-label treatment with Fabrazyme. Podocytes and epithelial cells of the distal convoluted tubules responded with modest reductions in GL-3 although neither cell type is thought to be primarily responsible for renal functional decline in Fabry patients. For other kidney cell-types, Fabrazyme reduced GL-3 levels, albeit not to zero.

(For more detail, refer to Section 4.2.1.1 and Section 4.2.1.2.)

3.) Heart and Skin Interstitial Capillary Endothelial Cells

Highly statistically significant ($p < 0.001$) improvement was also obtained in the clearance of GL-3 in the interstitial capillary endothelial cells of the heart and skin following Fabrazyme treatment in the Phase 3 Placebo-Controlled Study which was again confirmed in the patients that crossed over from placebo to Fabrazyme treatment during the Phase 3 Extension Study. Repeat skin biopsies have been obtained every 6 months through 18 months and then yearly thereafter, in the Phase 3 Extension Study; continued efficacy has been seen through 18 months of this extension study as assessed by GL-3 clearance to normal or near normal levels in 41/46 (89%) patients at the 18 month timepoint. Four of the five patients that had non-zero skin scores at 18 months underwent subsequent biopsies and now have zero scores. (For more detail, refer to Section 4.1.2.1.)

4.) Pain

Using the Short Form McGill Pain Questionnaire, there were significant improvements in multiple pain parameters within treatment groups in the Phase 3 Placebo-Controlled Study. This improvement has been maintained for up to 24 months of Fabrazyme therapy. However, there was no significant difference between treatment groups in pain parameters, possibly due to a strong placebo effect. (For more detail, refer to Section 4.1.2.3)

5.) Urinary and Kidney Tissue GL-3 Levels

For GL-3 levels in urine and kidney tissue, the percent change was compared between treatment groups using the rank sum score for each patient. There was a statistically significant difference in the distribution of rank sum scores between the two treatment groups ($p = 0.003$). This indicates an improvement in this composite endpoint. (For more detail, refer to Section 4.1.2.2.)

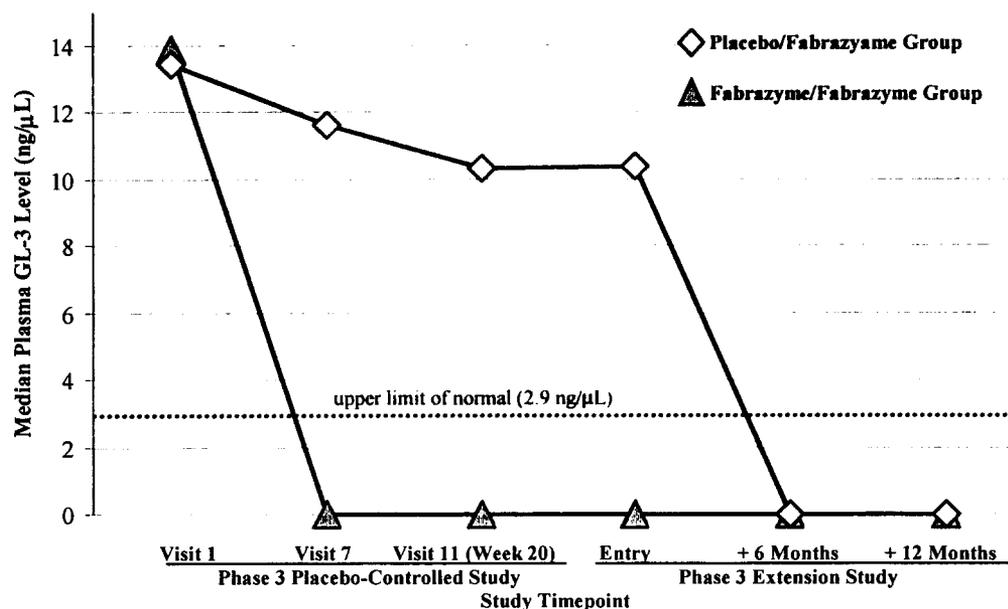
6.) Plasma GL-3

Plasma GL-3 levels were measured (by ELISA) as an indicator of α GAL activity and an indirect marker of the level of Fabrazyme reaching the different cells with accumulated GL-3. For A highly statistically significant difference was observed between the two treatment groups in percent change from Baseline to Week 20 for plasma GL-3 ($p < 0.001$). Median plasma GL-3 levels are presented in Figure 2. In the Fabrazyme treatment group, median plasma GL-3 levels decreased by 100% from Baseline to Week 20, while the median value in the placebo treatment group decreased by 16.5%.

Plasma GL-3 levels obtained for patients who originally received placebo in the Phase 3 Placebo-Controlled Study and who then switched to Fabrazyme treatment in the Phase 3 Extension Study were similarly decreased to levels obtained for patients who originally received Fabrazyme in the Phase 3 Placebo-Controlled Study. Plasma GL-3 remains undetectable in most patients, although it has been seen to rise in patients who have missed infusions. In addition, a strong association

was observed between the median percent decrease from Baseline to Week 20 in plasma GL-3 levels and the reduction in kidney interstitial capillary endothelial cell histology scores.

Figure 2 Median Plasma GL-3 Levels in the Phase 3 Placebo-Controlled Study and Phase 3 Extension Study



Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

(For more detail, refer to Section 4.1.3.1 and Section 4.2.1.3.)

7.) Renal function

Average renal function as assessed by serum creatinine was normal in both groups at Baseline and did not change significantly in either treatment group during the Phase 3 Placebo-controlled study and for 24 months into the extension study (i.e., up to ~30 months total treatment with Fabrazyme). (Table 1) (For more detail, refer to Section 4.1.3.3 and Section 4.2.1.4.)

Table 1 Mean (±SD) Serum Creatinine Levels (mg/dL)

Treatment Group	Phase 3 Placebo-Controlled Study		Phase 3 Extension Study			
	Baseline	Week 20	6 Months	12 Months	18-Months	24-Months
Placebo/Fabrazyme	0.8 ± 0.2 (n = 29)	0.8 ± 0.3 (n = 29)	0.8 ± 0.3 (n = 26)	0.8 ± 0.3 (n = 28)	0.9 ± 0.3 (n = 27)	0.8±0.4 (n = 25)
Fabrazyme/Fabrazyme	0.8 ± 0.2 (n = 29)	0.9 ± 0.2 (n = 29)	0.9 ± 0.2 (n = 28)	0.9 ± 0.3 (n = 28)	0.9 ± 0.3 (n = 28)	0.9±0.4 (n = 25)

Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

8.) Quality of Life as Measured by the SF-36 Health Status Survey

The mean change in the scores was calculated for the eight categories and the combined categories that comprise the physical and mental component scales. Improvements in both treatment groups were observed for several parameters across both populations, although between-group differences were not statistically significant.

(For more detail, refer to Section 4.2.1.6.)

9.) Phase 2 Open-Label Study (Japan)

An open-label, bridging study that adhered to the ICH Harmonized Tripartite Guideline (Feb 5, 1998) was completed in Japan on 13 patients. Entry criteria and endpoints were virtually identical to the Phase 3 Placebo-Controlled Study described above. This study confirmed, and extended, the findings of the Phase 3 Study. The same significant reductions of GL-3 to normal or near normal were seen in multiple cell types in the kidney following 20 weeks of Fabrazyme treatment. Similarly, plasma GL-3 levels became undetectable and renal function was preserved. These findings further confirmed the results of the larger Phase 3 Placebo-Controlled Study.

(For more detail, refer to Section 4.3.)

Safety

Full safety data derived from the treatment of 71 patients in three major studies (Phase 3 Placebo-Controlled Study, Phase 3 Extension and Phase 2 Open-Label [Japan]) demonstrate that patients are able to tolerate the long-term use of Fabrazyme. To date, over 350 patients have received over 4000 total infusions of Fabrazyme (including patient experience with Fabrazyme through compassionate use/special access, other on-going studies and post-approval commercial use in Europe and elsewhere) with the longest patient on therapy for over 3 years.

In the Phase 3 Placebo-Controlled Study, a statistically significant difference was observed for three adverse events that were reported more frequently in patients treated with Fabrazyme compared to placebo. These adverse experiences were rigors (15/29 [52%] vs. 4/29 [14%], $p=0.004$), fever (14/29 [48%] vs. 5/29 [17%], $p=0.024$), and skeletal pain (6/29 [21%] vs. 0/29 [0%], $p=0.023$) in the Fabrazyme vs. placebo treatment groups. Skeletal pain reported during this study represents isolated musculoskeletal events and is most likely not due to the infusion of Fabrazyme. Rigors and fever represent primarily infusion-associated reactions. The initial presentation of these most often coincided with IgG seroconversion. When rigors and fever occurred, they were generally mild to moderate in nature and were successfully managed by a temporary reduction in infusion rate, and treatment with acetaminophen and diphenhydramine. (For more detail, refer to Section 4.5.1.1.)

The majority of patients develop IgG antibodies during treatment. Among IgG positive patients in Phase 3 Placebo-Controlled Study whose antibody data have been followed for approximately 24-30 months in Phase 3 Extension Study, almost half have experienced a four-fold reduction in titer with continued treatment. Similar findings have been observed in patients enrolled in the Phase 2 Open-Label Study (Japan). Immune tolerance was achieved in seven patients as of the 24 month timepoint of the Phase 3 Extension Study. The proportion of patients who IgG seroconverted is almost identical in the Fabrazyme patients in the Phase 3 Placebo-Controlled (83%) and Phase 2 Open-Label study (Japan) (85%). IgG seroconversion does not impact efficacy as assessed by i) sustained clearance of tissue GL-3 as measured by light microscopy; ii) sustained clearance of plasma GL-3; and iii) stable renal function. (For more detail, refer to Section 4.5.3.1 and Section 4.5.3.2.)

There have been no reports of anaphylaxis. Of over 350 patients treated with Fabrazyme, two patients tested serum IgE positive and three patients tested skin test positive representing < 1.4% of all treated patients. One patient has been successfully rechallenged repeatedly with commercial product, three patients have been treated under Genzyme's cautious graded rechallenge protocol (AGAL-019-01), and the fifth patient is pending rechallenge. (For more detail, refer to Section 4.5.3.5.)

Long-term treatment has demonstrated a progressive decrease in the number of patients with infusion-associated reactions. In addition, long-term data have demonstrated that patients are able to successfully tolerate infusion rate increases resulting in a reduction in the total infusion time. (For more detail, refer to Section 4.5.1.2.)

Results from laboratory tests are similar across all studies and indicate that treatment with Fabrazyme appears to have no toxic effect. Chronic therapy is generally well tolerated. (For more detail, refer to Section 4.5.4.)

Phase 4 Clinical Program

Genzyme has requested an accelerated approval pursuant to 21 CFR Part 601, Subpart E. These regulations provide a mechanism by which products that offer meaningful therapeutic benefits over existing treatments for serious, life-threatening illnesses are made available (marketing approval granted) as soon as possible based on demonstrated effects on surrogate endpoints reasonably likely to predict clinical benefit. Approval under this regulation is subject to the requirement that the applicant study the product further to verify and describe the clinical benefit.

Genzyme has conducted, completed and reported the results of a Phase 3 placebo-controlled, adequate and well-controlled clinical study and established that Fabrazyme has an effect on a

surrogate endpoint that is reasonable likely, on the basis of pathophysiologic evidence, to predict a clinical benefit. It could be argued that the submitted evidence goes beyond a surrogate endpoint and is tantamount to clinical evidence. Nonetheless, since the BLA was submitted under the accelerated approval mechanism, Genzyme has fully enrolled a Phase 4 Study (currently on-going) and therefore meets the requirements for accelerated approval in that the confirmatory study is underway.

In this section, we describe the ongoing Phase 4 study as well as important modifications to the Phase 4 Program that we have proposed to FDA to address their concerns. Genzyme wants to stress that the FDA's concerns currently preventing approval of Fabrazyme are addressable in various ways. More importantly, these are the types of issues that can and should be resolved in a post-approval setting as intended by the Accelerated Approval regulations so that the very small but seriously ill patient population may be granted access to this important therapy for a disease where no alternative is available.

Current Phase 4 Clinical Study

Genzyme has designed, submitted and fully enrolled an adequate, well-controlled clinical trial (multicenter, randomized, double-blind, placebo-controlled) to verify and describe the clinical benefit. This placebo controlled study design was proposed in August 2000 because no alternatives existed at that time for meeting FDA's requirement that the study would be well-controlled. This study assesses the time to clinically significant progression of renal disease, cardiac disease, cerebrovascular disease, and/or death in patients with advanced Fabry disease. Patients are assigned to treatment using a 2:1 (Fabrazyme:Placebo) randomization scheme. Patients assigned to either treatment group will continue to receive standard care and are monitored closely for progression of disease. If patients manifest clinically significant progression, they are to receive open-label treatment with Fabrazyme. It is anticipated that the total duration of this study will be approximately 3 years.

Extensive resources have been devoted to this Phase 4 study and much progress has been made. It is currently fully enrolled, with the first patient having received treatment for approximately 21 months. Over 235 patients have been consented and screened at 34 different sites worldwide. Seventy-six patients have met enrollment criteria and have been randomized and infused.

In several discussions between Genzyme, FDA, investigators, and other experts, concerns have been raised about several aspects of this Phase 4 study. These have included concerns about the ethics and feasibility of completing a long-term placebo-controlled study in a post-marketing setting with an endpoint of irreversible organ damage, and concerns that the post-marketing study might yield inconclusive results creating uncertainty as to the clinical benefit and possible

withdrawal of the product that might, in fact be beneficial. Therefore, Genzyme has developed an alternative proposal to meet the accelerated approval requirements for collecting data from adequate and well-controlled post-marketing studies to verify and describe the clinical benefit of Fabrazyme (referred to Proposed Phase 4 Program). (For more detail, refer to Section 6.2.)

Proposed Phase 4 Program

Genzyme is proposing a modified and expanded Phase 4 program and has already made significant progress in its implementation. This modified Phase 4 program would include extensive study of the impact of Fabrazyme on a broader cross-section of the Fabry population than is currently being studied in the Phase 4 study. This new four-pronged program which is described below will eliminate the concerns regarding feasibility of patient retention in a post-marketing setting and obviate the ethical concerns regarding a placebo-controlled, post-marketing study in which the endpoint will likely result in irreversible end organ damage. It also includes several extensive studies of the clinical impact of Fabrazyme and provides added assurance against the possibility of inconclusive results.

1. Develop a prospectively defined, comprehensive natural history database.

- The collection of these data under a prospectively defined protocol is complete. The final study report was submitted to the BLA on October 18, 2002. The natural history database consists of 447 unique patients from 27 sites in 5 countries. The data from the Historical Study are recent, with 75% of the serum creatinine measurements in the “qualified patients” occurring after April 1996. The contemporaneous nature of the historical data suggests that statistical models can be utilized that would provide a robust assessment of the data. (See Section 6.3 for further details and results).

2. Utilize an appropriate subset of patients from the natural history database as a control group to convert the current Phase 4 Placebo-Controlled study into a single-arm, historical-controlled study by comparing the renal disease event rates in this relatively advanced disease population.

- The proposed protocol (Genzyme Study AGAL-008-00) for the conversion of the study was submitted to FDA in April 2002. The protocol synopsis is included in Appendix 8.1.
- Whereas the current placebo-controlled study focused on a composite endpoint consisting of renal, cardiac, cerebrovascular, and death components, the focus of the active treatment protocol will be improvement in renal outcomes.

- Converting the placebo-control design to a single-arm, active treatment design obviates any concerns about the feasibility or ethics of maintaining Fabry patients on placebo in a post-marketing setting and allows the study to focus on progression of renal disease, the most common devastating manifestation of Fabry disease.
 - Appropriate statistical methodology can be utilized to analyze the outcomes in the Fabrazyme treated and historical control Fabry patients. (See below and Sections 6.4.1 and 6.4.2 for further details.)
- 3. Utilize an appropriate subset of patients from the natural history database to compare the event rate of renal disease in the 58 Fabry patients enrolled in the Phase 3 Extension study (Genzyme Study AGAL-005-99).**
- The patients in the Phase 3 Extension study represent patients with much less advanced renal disease.
 - The patients in the Phase 3 Extension Study will be followed for at least 5 years from the date of enrollment in the Phase 3 Placebo-Controlled Study.
 - The interim analysis of the Phase 3 Extension Study patients after 24-30 months on Fabrazyme therapy was completed in November, 2002. The interim analysis shows encouraging trends with respect to slowing the progression of renal functional decline in patients receiving Fabrazyme for up to 30 months compared to the comparable historical database of untreated Fabry patients (See Section 4.2.1.5)
- 4. Commit to an extensive Fabry Registry program (already in place worldwide)**
- The Fabry Registry will be open to any Fabry patient regardless of treatment and is intended to collect long-term data with the express purpose of expanding the knowledge of Fabry disease and treatment with Fabrazyme.

This multi-faceted approach provides additional confidence and obviates concerns regarding inconclusive results from any one element. (For more detail, refer to Section 6.)

Statistical Methodology

Statistical methodologies exist to derive appropriate control groups from the historical database and to compare the control groups to the active treatment groups in the Phase 3 Extension and Phase 4 studies with regard to renal event rates. For use as a control group for the Phase 4 trial, estimates of renal event rates were obtained from the appropriate subset of patients in the historical database by modeling the underlying patient-specific trends in serum creatinine over the duration of follow-up using linear random effects modeling. The renal event rate estimate

was shown to be robust following supplementary analyses. Further additional analyses were conducted in response to FDA questions to support the methodology and results. All of the modeling scenarios thus conducted are remarkably consistent. See Section 6.4.1 for more detail.

To further address FDA concerns regarding issues such as the representativeness of the historical data base and the missing/non-uniformly distributed serum creatinine values from the historical data base Genzyme has also pursued alternative methods of analysis. The alternative methodology (developed by Professor Donald Rubin of Harvard University) consists of matching the historical control patients to the clinical study patients using propensity scoring algorithms. Multiple imputation will be used to fill in the missing data. The imputation will be done by using data from the two control groups, placebo and historical. Following imputation, the data from the randomized patients will be compared to the matched historical control patients by logistic regression using covariates to be discussed with FDA. For more detail, refer to Section 6.4.2.

Justification of Historical Control Phase 4 Study Proposal

The purpose of conducting clinical investigations is to distinguish the effects of a drug from other influences such as spontaneous change in the course of the disease, placebo effect, or biased observations. An adequate and well-controlled study has several characteristics one of which is that the design must permit a valid comparison with a control group in order to provide an adequate assessment of drug effect. The FDA and ICH recognize historical controlled studies in the hierarchy of clinical study designs. The proposal to convert the current Phase 4, placebo-controlled clinical study into a single-arm, historical-controlled study is supported by the ICH Guideline, E-10 (Choice of Control Group and Related Issues in Clinical Trials).

There have been cautions raised about the use of historical controlled efficacy studies with regard to such points as whether disease progression can be characterized sufficiently, whether there are differences in diagnoses, follow-up, frequencies of measurements, concomitant medications or treatment paradigms. However, historical controlled studies have been used successfully in settings in which the disease is rare, there is a high likelihood of a particular outcome in the absence of therapy and the new intervention can produce dramatic results. These conditions apply in the setting of enzyme replacement therapy for patients with Fabry Disease.

Genzyme is not proposing the single-arm, historical-controlled trial in a vacuum to demonstrate safety and effectiveness of Fabrazyme for the prevention of progression of renal impairment in Fabry patients. Genzyme has conducted and reported the unequivocal results of the pivotal Phase 3 placebo-controlled clinical trial demonstrating the safety and effectiveness of Fabrazyme as determined on a histologically assessed and related renal vascular endpoint. The proposed single-arm, historical-controlled trial is a confirmatory study to verify and describe the clinical

benefit that will likely be derived on the basis of the effect of enzyme replacement therapy on the histopathology of the disease. When one considers the extremely rare nature of the disease, the existing clinical and preclinical data attesting to the effect of enzyme replacement therapy on the underlying histopathology and the inherent complexities of conducting long-term (35 month) placebo-controlled trials in this severely ill patient population, the proposed Phase 4 program represents a flexible and robust approach. (For more detail, refer to Section 6.2)

Conclusions

Currently, no treatment is available for the prevention or stabilization of the progressive vascular damage and resultant end organ destruction of Fabry disease. The GL-3 accumulation in multiple cell types, but particularly the endothelial cells, leads to the high morbidity and mortality rates due to renal failure, stroke, and cardiovascular disease.

Fabrazyme therapy has been shown to clear GL-3 to normal or near-normal levels in critical cells involved in the pathophysiology of the disease, including the capillary endothelium, and thus has the potential for long-term clinical benefit. This is further supported by trends in patients receiving Fabrazyme for up to 30 months where data indicate a slowing in progression of renal functional decline compared to a matched historical database of untreated Fabry patients. The data demonstrate that Fabrazyme is well-tolerated and has a robust efficacy profile.

The ongoing Phase 4 study (fully enrolled, with the first patient having received treatment for approximately 21 months) can now be converted to a historical control study based on the significant historical data that have been collected. This, along with the additional aspects of the proposed expanded Phase 4 program, ensure that data will be collected in the post-marketing setting to demonstrate the long-term clinical benefit of Fabrazyme. Thus the requirements under accelerated approval for adequate and well-controlled post-marketing studies to be underway at the time of approval of the BLA have been met.

At this time, the data available indicate that Fabrazyme is an effective treatment for patients with Fabry disease. Therefore, patients should not be denied the opportunity of this therapy, and approval is warranted at this time.

Based on the data presented, the proposed **label indication** is:

Fabrazyme® (agalsidase beta) is indicated for use as a long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease. Fabrazyme® treats the underlying pathology of Fabry disease by significantly clearing globotriaosylceramide (GL-3) to normal or near normal levels from the vascular endothelium of the kidney, heart and skin. Clearance was also demonstrated in other critical cell types, such as mesangial cells, glomerular capillary endothelium, interstitial cells and non-capillary endothelium, and reduced in cell types with the highest substrate burden (vascular smooth muscle cells, tubular epithelium and podocytes).

Table of Contents

1.	INTRODUCTION	22
1.1	Proposed Indication and Usage.....	22
1.2	Fabry Disease: Molecular Basis and Epidemiology	22
1.3	Clinical Manifestations and Natural History/Clinical Course of Fabry Disease	23
1.4	Pathologic Basis of Fabry Disease.....	25
1.5	Treatment of Fabry Disease	26
1.6	Treatment of Fabry Disease with Fabrazyme®	27
1.7	Regulatory Status of Fabrazyme	27
1.8	Product Description	27
2.	NONCLINICAL DEVELOPMENT PROGRAM	28
2.1	Summary of Preclinical Pharmacology and Toxicology Studies.....	28
2.1.1	Pharmacodynamics	28
2.1.2	Pharmacokinetics	29
2.1.3	Toxicology	30
2.1.4	Reproduction Studies.....	30
2.1.5	Mutagenicity/Carcinogenicity.....	30
2.1.6	Dosage Regimen.....	31
2.1.7	Conclusions.....	31
3.	FABRAZYME® CLINICAL DEVELOPMENT PROGRAM	32
3.1	Clinical Pharmacology.....	33
3.1.1	Pharmacokinetics Results of the Phase 1/2 Study and Phase 3 Placebo-Controlled Study	33
3.1.2	Pharmacodynamics and Dose Response.....	35
3.2	Clinical Efficacy – Phase 3 Placebo-Controlled Study.....	36
3.2.1	Selection of Treatment Dose and Regimen	36
3.2.2	Selection of Endpoints	36
3.2.2.1	Considerations in Selection of Appropriate Study Endpoints.....	36
3.2.2.2	Selection of the Primary Endpoint: GL-3 Clearance	38
3.2.3	Study Design.....	39
3.2.3.1	Estimation of Sample Size.....	41
3.2.3.2	Evaluation of the Primary Endpoint and other Histologic Endpoints	41
3.2.3.3	Secondary, Tertiary, and Other Endpoints.....	45
3.2.3.4	Additional Histologic Analyses.....	45
4.	CLINICAL EFFICACY AND SAFETY	47
4.1	Phase 3 Placebo-Controlled Study.....	47
4.1.1	Primary Endpoint Results – Phase 3 Placebo-Controlled Study	47
4.1.1.1	Primary Endpoint Sensitivity Analysis	50
4.1.2	Secondary Endpoints	51

4.1.2.1	Capillary Endothelium of the Kidney, Skin, and Heart	51
4.1.2.2	Urinary and Kidney Tissue GL-3 Levels	53
4.1.2.3	Pain Measured by the Short Form McGill Pain Questionnaire	53
4.1.3	Tertiary and Other Endpoints	55
4.1.3.1	Plasma GL-3	55
4.1.3.2	Relationship Between Biochemical and Histologic Clearance of GL-3	56
4.1.3.3	Renal Function	58
4.2	Phase 3 Extension Study	58
4.2.1	Study Results	59
4.2.1.1	Histologic Assessment of Interstitial capillary endothelial Cells of Kidney, Skin, and Heart	59
4.2.1.2	Histologic Assessment of Additional Cell-Types of the Kidney and Skin	63
4.2.1.3	Plasma GL-3	67
4.2.1.4	Renal Function	68
4.2.1.5	Progression of Renal Disease in Patients Treated with Fabrazyme Compared to an Untreated Historical Control Population of Patients with Fabry Disease	71
4.2.1.6	Quality of Life as Measured by the SF-36 Health Status Survey	72
4.3	Phase 2 Open-Label Study (Japan)	73
4.4	Discussion of Clinical Relevance and Summary of Results	74
4.4.1	Discussion of Clinical Relevance of Results	74
4.4.2	Summary of Efficacy Results	77
4.5	Clinical Safety	78
4.5.1	Adverse Events	79
4.5.1.1	Most Common	79
4.5.1.2	Infusion-Associated Reactions (IAR)	84
4.5.2	Serious Adverse Events	87
4.5.2.1	Deaths	87
4.5.2.2	Other Serious Adverse Events (SAE)	88
4.5.2.3	Serious Adverse Events – Other Studies/Programs	93
4.5.2.4	Study Discontinuations	97
4.5.3	Immunogenicity	98
4.5.3.1	Seroconversion Rates	98
4.5.3.2	No Impact of Seroconversion on Efficacy	99
4.5.3.3	Immune Complexes	100
4.5.3.4	Change in Antibody Titers Over Time	100
4.5.3.5	IgE and Experience with Fabrazyme Rechallenge	102

4.5.4	Laboratory And Other Test Abnormalities	103
4.5.5	Summary of Safety.....	103
4.5.6	Safety Conclusion	104
5.	RISK/BENEFIT	105
5.1	Fabry Disease and Current Medical Care	105
5.2	Fabrazyme Benefits.....	105
5.3	Risks Associated with Fabrazyme Therapy	105
5.4	Conclusions.....	106
6.	PHASE 4 POST-APPROVAL CLINICAL PROGRAM TO VERIFY CLINICAL BENEFIT	107
6.1	Current Phase 4 Study (Genzyme Study AGAL-008-00).....	108
6.1.1	Status of Current Study.....	109
6.1.2	Current Study Design Issues	109
6.2	Proposal for New Phase 4 Clinical Program.....	110
6.2.1	Rationale for Historical Control Phase 4 Study.....	112
6.3	Epidemiological Study of the Natural History of Fabry Disease.....	113
6.3.1	Study Methodology.....	113
6.3.2	Data Collected.....	114
6.4	Creation of Fabry Patient Historical Control Groups (from Natural History Database) for Comparison to Patients in the Phase 3 and Phase 4 studies	114
6.4.1	Linear Random Effects Models	115
6.4.2	Assessing the Efficacy of Fabrazyme in a Single Arm Phase 4 study by using a Matching historical Control Study Group using Propensity Scoring Algorithms	118
6.4.2.1	Analysis Methodology Summary	119
6.5	Phase 4 Summary.....	121
7.	CONCLUSION	123
8.	APPENDICES	124
8.1	Proposed Phase 4 Single Arm Protocol Synopsis.....	125
8.2	Published Results of Clinical Studies with Fabrazyme	130
8.3	Final Report: Epidemiological Study of Fabry Disease.....	164
8.4	Statistical Analysis Plan: Matched Historical Control Methodology	248
9.	SELECTED REFERENCES	273

Glossary of Selected Terms Used in Reporting on the Phase 3 Placebo-Controlled Study and the Phase 3 Extension Study

Study Timepoints

In order to differentiate between the beginning of the Phase 3 Placebo-Controlled Study and the beginning of the Phase 3 Extension Study:

- “**Baseline**” was used to identify the beginning timepoint of the Phase 3 Placebo-Controlled Study and
- “**Entry**” was used to identify the beginning timepoint of the Phase 3 Extension Study

For the Phase 3 Placebo-Controlled Study, major Efficacy and Safety measurements were conducted at **Baseline** and at **Week 20** (Final Study Visit).

For the Phase 3 Extension Study, major Efficacy and Safety measurements were conducted every 6 months (i.e., + **6, 12, and 18 months**) relative to the **Entry** timepoint.

Study Treatment Groups

Placebo/Fabrazyme (PL/FZ) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme (FZ/FZ) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

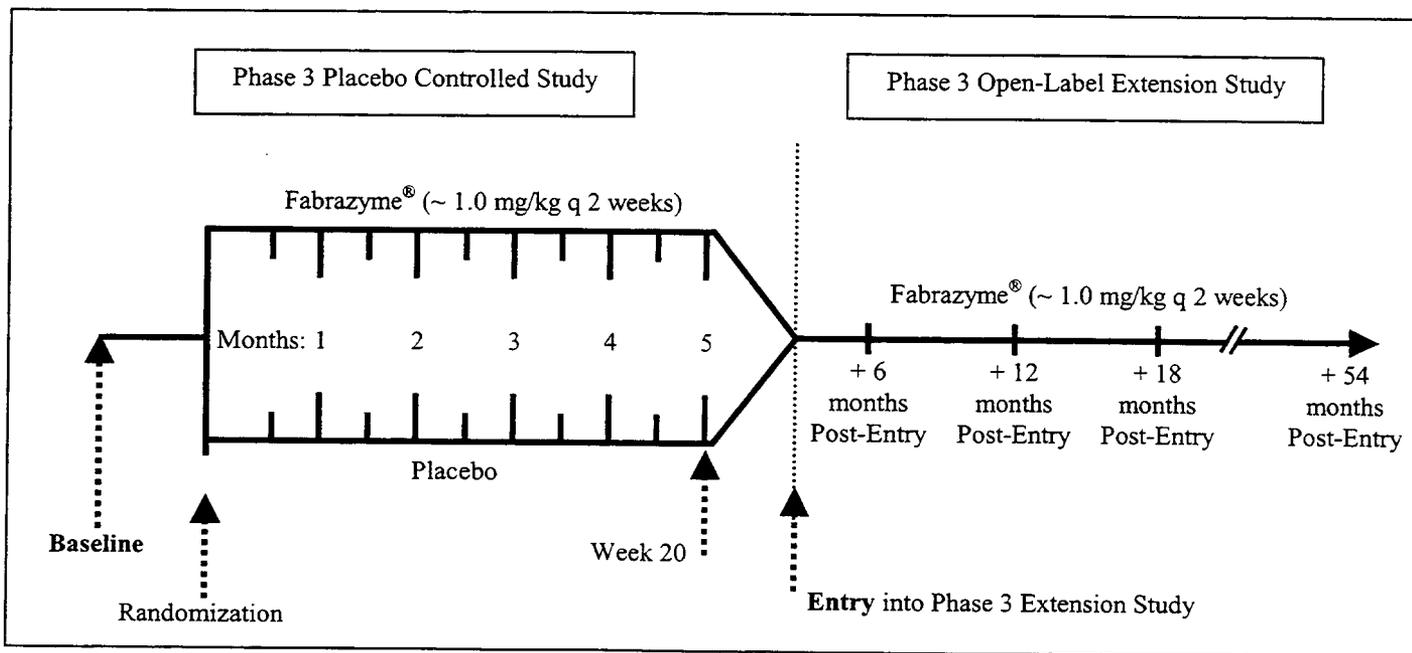
Efficacy and Safety Reporting

For the Phase 3 Placebo-Controlled Study, major Efficacy and Safety measurements are reported relative to **Baseline**.

For the Phase 3 Extension Study, major Efficacy and Safety measurements are reported relative to Baseline and to **Entry**.

The difference between **Baseline** and **Entry** becomes most important when considering the time on treatment for the patients in the Phase 3 Extension Study. In the Phase 3 Extension Study **Placebo/Fabrazyme** patients have received approximately 20 fewer weeks of treatment with Fabrazyme than the **Fabrazyme/Fabrazyme** patients.

Study Outline



1. INTRODUCTION

1.1 Proposed Indication and Usage

Fabrazyme® (agalsidase beta) is indicated for use as a long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease (α -galactosidase-A (α GAL) deficiency). Fabrazyme treats the underlying pathology of Fabry disease by significantly clearing globotriaosylceramide (GL-3) to normal or near normal levels from the vascular endothelium of the kidney, heart and skin. Clearance was also demonstrated in other cell types, such as mesangial cells, glomerular capillary endothelium, interstitial cells and non-capillary endothelium, and reduced in cell types with the highest substrate burden (vascular smooth muscle cells, tubular epithelium and podocytes).

1.2 Fabry Disease: Molecular Basis and Epidemiology

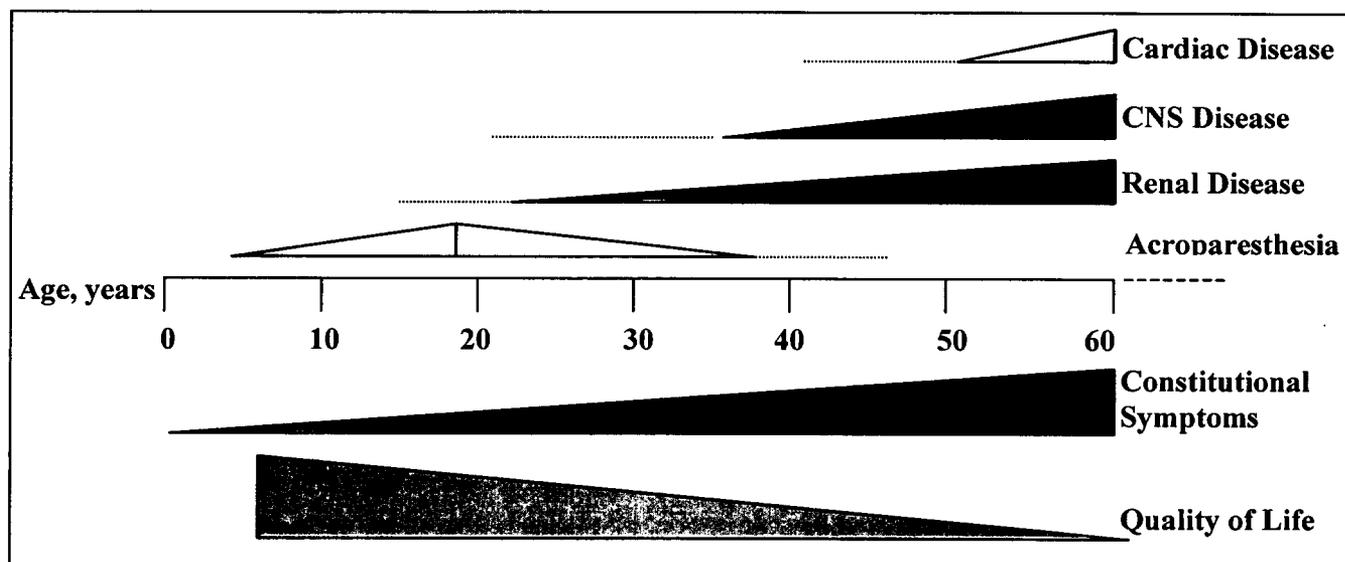
Fabry disease (also referred to as Anderson-Fabry disease) is an X-linked inborn error of metabolism characterized by markedly decreased or absent activity of the lysosomal hydrolase, α -galactosidase-A (α -GAL). Fabry disease is a panethnic, monogenic disorder. The underlying cause of Fabry disease is a mutation in the nucleotide sequence coding for the structural portions of α GAL. It is estimated that the incidence of Fabry Disease is 1:40,000 males (*Desnick, 2001, in The Metabolic Basis of Inherited Disease*) thus the patient population in the United States is approximately 3500 males (*United States Government Census, 2000*). Deficiency of α GAL leads to the progressive accumulation of glycosphingolipids, predominantly globotriaosylceramide (GL-3) in the lysosomes of multiple cell-types in many tissues, including endothelial, perithelial, and smooth-muscle cells of blood vessels, ganglion cells of the autonomic nervous system, cardiomyocytes of the heart, epithelial cells of glomeruli and tubules in the kidney, and epithelial cells of the cornea (*Desnick, 1995, in The Metabolic Basis of Inherited Disease*). These pathologic accumulations begin in utero (*Desnick, 2001, in The Metabolic Basis of Inherited Disease*) and continue to progress throughout life.

Accumulation of GL-3 in lysosomes of affected individuals leads to the characteristic pathologic cellular inclusions of Fabry disease. These inclusions appear as distinct granules stainable by lipophilic dyes under light microscopy and as osmophilic lamellated whorls (myelin figures) inside lysosomes under electron microscopy. The chemical nature of these inclusions may be demonstrated in situ by binding of agents such as verotoxin (*Lingwood et al., 1987, J Biol Chem*) (*Zeidner, et al., 1999, Analytical Biochemistry*) and by a characteristic retention time after chemical modification by high-pressure liquid chromatography (HPLC) (*Oshima, et al., 1990, Biochim Biophys Acta*) (*Ullman, et al., 1980, Clin Chem*).

1.3 Clinical Manifestations and Natural History/Clinical Course of Fabry Disease

Although abnormal glycosphingolipid accumulation begins in utero, sufficient pathologic deterioration leading to clinical manifestations typically does not become evident for years-to-decades. However, the ultimate clinical consequences of years of tissue GL-3 accumulation are progressive impairment of tissue and organ function. More specifically, patients suffering from Fabry disease have high morbidity and mortality rates due to renal failure, stroke, and cardiovascular disease. Other common features of Fabry disease are pain, dysfunction of the autonomic nervous system, hypohidrosis, and corneal opacities. Patients may also present with gastrointestinal symptoms such as indigestion, cramps, gastric reflux, and bouts of diarrhea. Figure 1-1 illustrates the disease course and body system involvement over time.

Figure 1-1 Illustration of Fabry Disease Timeline



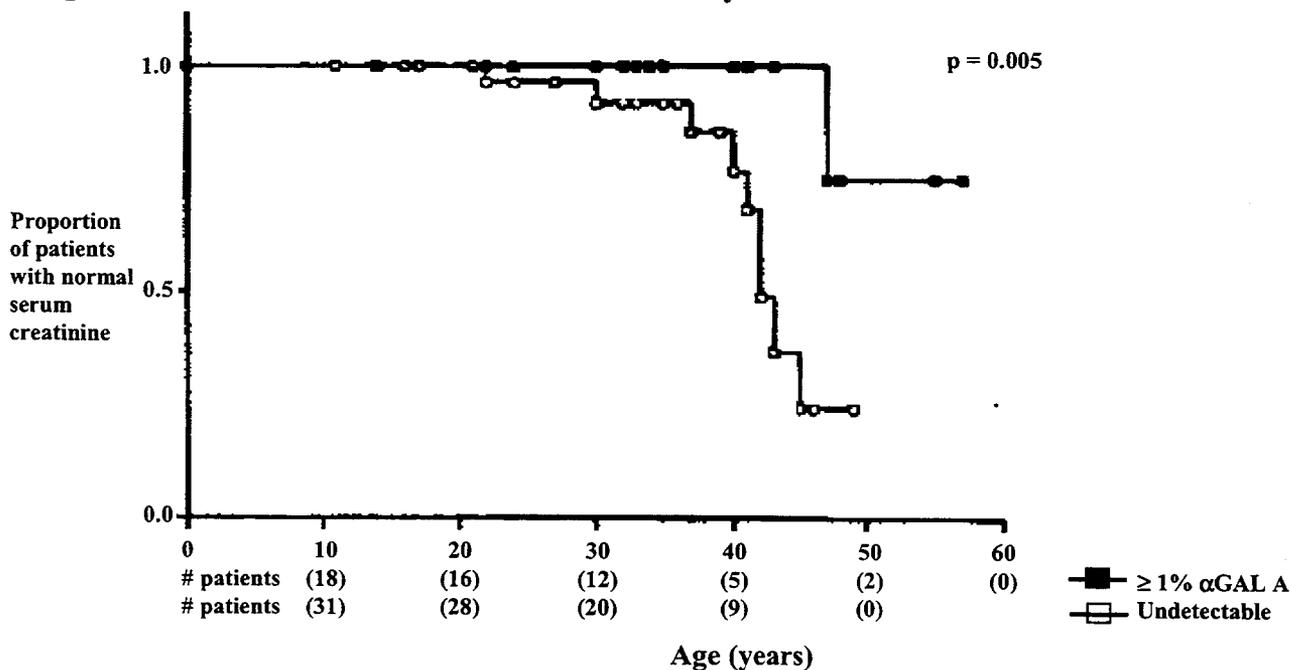
The clinical course and organ involvement associated with the disease are heterogeneous, varying with gender, age, and genotype. Additionally, the rates of progression of specific clinical manifestations are incompletely understood. Specifically, since this is an X-linked genetic disorder, affected males have much less residual enzyme activity than females. Males, therefore, are generally affected more severely and earlier than females. However, depending on the nature of random X-inactivation (lyonization), females have variable and, sometimes, significant clinical manifestations. Extent of disease appears to correlate with the degree of GL-3 tissue accumulation. While no precise genotype/phenotype correlation has been established at present, patients with certain mutations have more residual enzyme activity (4 – 14% of normal). Such

patients manifest a milder form of the disease, known as the “cardiac variant” (see Section 1.4), which presents later in life with a distinct pathology and clinical course.

Although the clinical course of Fabry disease is variable, “classic” Fabry patients who have little (<1%) or no enzyme activity typically:

- develop acroparesthesias as early as 5-7 years of age as the initial symptom (*Gordon, 1995, Pediatr Neurol*) (*Shelley, 1995, Pediatr Dermatol*). However, acroparesthesias often disappear or are reduced in frequency and severity in others, presumably as nerve fibers die;
- present with the onset of renal insufficiency in the fourth or fifth decade of life and progress to end-stage renal disease requiring dialysis and/or transplantation (Figure 1-2). Renal insufficiency occurs in the vast majority of patients and is the major cause of morbidity and mortality in this disease (*Meroni, et al., 1997, Contrib Nephrol*) (*Tsakiris, et al., 1996, Nephrology Dialysis Transplantation*);

Figure 1-2 Onset of Chronic Renal Insufficiency: Effect of Level of α-Galactosidase A



The probability of normal creatinine (< 1.5 mg/dL) is expressed as a function of age in Fabry patients with undetectable levels of αGAL A activity (open square) and in Fabry patients with ≥ 1% αGAL A activity (closed square). Age of onset of chronic renal insufficiency was significantly later in patients with detectable αGAL A activity (p = 0.005). (*Branton, 2002, Medicine*)

- develop cerebrovascular complications during the fourth and subsequent decades (*Crutchfield et al., 1998, Neurology*) (*Mitsias and Levine, 1996, Ann Neurol.*) (*Grewal, 1994, J Neurol*) and demonstrate abnormal autonomic responses throughout the course of the disease;

- develop a concentric hypertrophic cardiomyopathy that may clinically manifest in the third and subsequent decades of life as a consequence of progressive GL-3 accumulation in cardiomyocytes (*Ferrans, 1969, Am J Cardiol*) (*Yanagawa, 1988, Acta Paediatr Jpn*) (*Goldman, 1986, J Am Coll Cardiol*) (*Bass, 1980, Am Heart J*);
- experience ischemic cardiac events secondary to endothelial dysfunction and small vessel occlusion (*Gubler, 1978, Kidney Int*) (*Desnick, 1995, in The Metabolic Basis of Inherited Disease*); and
- experience a progressive increase in the frequency and intensity of non-specific constitutional symptoms and a decrease in quality of life.

1.4 Pathologic Basis of Fabry Disease

While many cell-types demonstrate abnormal glycosphingolipid accumulation, it is important to note that the clinical manifestations of Fabry disease primarily result from pathologic GL-3 accumulation in the vascular endothelium of many tissues (*Crutchfield et al., 1998, Neurology*) (*Hasholt and Sorensen, 1986, Hum Genet*) (*Nakamura et al., 1981, Acta Derm Venereol*) (*Ohshima et al., 1999, Proc Natl Acad Sci USA*) (*Sakuraba et al., 1987, Clin Genet*) (*Schatzki et al., 1979, J Surg Pathol*) (*DeGraba, 2000, Ann Neurol*) (*Desnick, 1995, in The Metabolic Basis of Inherited Disease*).

Two “experiments of nature” provide evidence that endothelial pathology is primarily responsible for the clinical manifestations of the disease. There is a subset of Fabry patients with a cardiac variant form of the disease (*von Scheidt, 1991, N Engl J Med*) (*Elleder, 1990, Virchows Arch A Pathol Anat*) (*Ikari, 1992, Br Heart J*) (*Yoshitama, 2001, Am J Cardiol*). This variant is a milder form of the disease in which patients have higher residual levels of α -galactosidase A activity. These patients present with clinical manifestations much later in life, often between the sixth and seventh decades, compared to patients with the classical form of the disease who experience more significant clinical manifestations between the fourth and fifth decades. Clinical manifestations among cardiac variants are primarily related to cardiomyopathy. These patients do not typically develop renal insufficiency though they may have proteinuria. Importantly, these patients have residual enzyme activity (ranging from 4 – 14% of normal), do not have significant GL-3 accumulation in endothelial cells, but do have GL-3 accumulation in cardiac myocytes, kidney tubular epithelial cells and podocytes.

Similarly, a second subset of patients, female heterozygotes who rarely develop renal insufficiency, have been reported to have significant GL-3 accumulation in podocytes, but not in endothelial cells of the kidney (*Farge, 1985, Arch Pathol Lab Med*) (*Gubler, 1978, Kidney Int*) (*Marguery, 1993, Dermatology*) (*Rodriguez, 1985, Arch Pathol Lab Med*) (*Wuthrich, et al, Nephrol Dial Transplant 1998*) (*Grunfeld, et al, Contrib Nephrol*). In this regard, a very enlightening case was recently discovered by Nieto and Desnick (personal communication). They have identified a 41 year old woman with a family history of Fabry disease who had a lifelong

history of signs and symptoms commonly seen in classical male Fabry hemizygotes. These included acroparesthesias, angiokeratomas, corneal opacities, abdominal discomfort, fatigue, and tinnitus. She had nephrotic range proteinuria for several years and a serum creatinine of 3.0 mg/dL. A renal biopsy was performed which revealed significant GL-3 accumulations not only in tubular epithelium but also in interstitial capillary endothelial cells. Her echocardiogram showed normal left ventricular size and function and mild mitral regurgitation. Presumably as a result of an unfortunate random X-inactivation pattern, this patient had a deficiency in endogenous α GAL more closely resembling that typically seen in male hemizygotes, and as a result, she also had the pathological and clinical manifestations commonly seen in classical male hemizygotes. These observations support the concept that Fabry is a vascular disease caused by abnormal GL-3 accumulation in endothelial cells that leads to the renal failure, myocardial ischemia, strokes and transient ischemic attacks in patients with the classical form of Fabry disease.

The anatomic and pathologic basis of vascular endothelial dysfunction in Fabry disease has been well documented (*Crutchfield et al., 1998, Neurology*) (*Hasholt and Sorensen, 1986, Hum Genet*) (*Nakamura et al., 1981, Acta Derm Venereol*) (*Schatzki et al., 1979, J Surg Pathol*) (*DeGraba, 2000, Ann Neurol*). As a consequence of the excessive accumulation of GL-3 deposits in the vascular wall, small capillaries collapse, thrombi replace endothelial cells and weakened vessels may give rise to telangiectatic eruptions (*Nakamura et al., 1981, Acta Derm Venereol*). Additionally, investigators have more recently surveyed Fabry patients for markers of endothelial cell injury and activation (ICAM-1, VCAM-1, P-selectin), leukocyte activation (CD11b) and coagulation (tPA, vWF, PAI). Abnormalities in these parameters in Fabry patients compared to controls suggest that the vascular endothelial cells of Fabry patients are in a chronic proinflammatory and prothrombotic state (*Sakuraba, 1987, Clin Genet*) (*DeGraba, 2000, Ann Neurol*). Ultimately, the disease leads to vascular insufficiency because of narrowing and thrombosis of small vessels. The resultant vascular insufficiency leads to peripheral neuropathy, and myocardial and cerebral infarction.

1.5 Treatment of Fabry Disease

Currently no specific treatment or cure exists for Fabry disease; therefore, therapy is aimed at sign and symptom palliation. However, the disease progression (renal, cardiac and cerebrovascular) is largely unaffected by current medical interventions. Pain management continues to be a large part of the medical therapy of Fabry disease. Opiates, diphenylhydantion, carbamazepine and gabapentin have all been used with varying degrees of success (*Lockman, 1973, Neurology*) (*Lenoir, 1977, Arch Franc Ped*) (*Filling-Katz, 1989, Neurology*) (*Inagaki, 1992, Brain Dev*) (*Desnick, 1995, in The Metabolic Basis of Inherited Disease*) (*Peters, 1997, Postgrad Med*). Oral anticoagulants and/or antiplatelet therapy are recommended for stroke-

prone patients. Dialysis and/or renal transplantation are the two currently available therapeutic options for end-stage renal disease.

1.6 Treatment of Fabry Disease with Fabrazyme®

Genzyme has developed Fabrazyme® containing the active ingredient recombinant human α -galactosidase A (r-h α GAL, agalsidase beta) as enzyme replacement therapy for patients with Fabry disease.

Fabrazyme is taken up by cells, at least in part by the mannose-6-phosphate receptor, and is transported to the lysosome. Hence, replacement therapy with Fabrazyme restores the missing enzyme activity and leads to the clearance of neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3) in the lysosomes of various cells that would, in Fabry disease, show pathologic accumulation. We have hypothesized that prolonged treatment with Fabrazyme resulting in removal of GL-3 accumulation will lead to stabilization or possible improvement in organ function.

1.7 Regulatory Status of Fabrazyme

Because of the serious, life-threatening nature of Fabry Disease and because it is believed that Fabrazyme could provide meaningful therapeutic benefits compared with existing palliative treatments, Genzyme and the Center for Biologics Evaluation and Research concurred that expedited development and marketing should be pursued in line with Fast Track status issued in 1999. In addition, due to the nature of the disease and the long, slow progression of organ failure, it was agreed that accelerated approval pursuant to 21 CFR Part 601, Subpart E was appropriate. This regulation provides for approval based on evidence from adequate and well-controlled clinical studies of the product's effect on a surrogate endpoint that is predictive of clinical benefit, followed by post-marketing studies to establish and confirm the degree of clinical benefit to patients.

Fabrazyme has been granted orphan drug status in the United States and the European Union (EU), among other regions. Fabrazyme has been approved for marketing in over 25 countries, including the European Union and Australasia.

1.8 Product Description

Recombinant-human α GAL is a highly purified recombinant form of the naturally occurring human glycoprotein and is produced in Chinese hamster ovary (CHO) cells transfected with a DHFR-vector carrying the cloned cDNA of the human α GAL-A gene. The biochemical characterization of Fabrazyme shows that the protein is richly sialyated and has sufficient exposed mannose-6-phosphate to allow appropriate lysosomal uptake.

2. NONCLINICAL DEVELOPMENT PROGRAM

The development strategy for Fabrazyme was based on the fact that it is derived from recombinant DNA technology [mammalian (CHO) cell culture], is identical to the natural form of the enzyme and would be inherently safe when administered to humans. The hamster cell expression system (CHO) was chosen for production of Fabrazyme because of the demonstrated safety profile over 20 years experience producing multiple therapeutic products using this system.

Studies were conducted for acute toxicity, long-term toxicity, and to assess the impact on fertility and reproductive development. No studies were conducted to assess oncogenic potential including clastogenicity. Additionally, detailed studies were conducted to evaluate the critical pharmacodynamic and pharmacokinetic properties of Fabrazyme. Results from all nonclinical pharmacology studies of Fabrazyme present no evidence of safety concerns at the dose of 1.0 mg/kg administered intravenously. The preclinical development plan took into consideration the International Conference on Harmonization S6 document entitled "Preclinical Safety evaluation of Biotechnology-derived Products".

The FDA Center for Biologics Evaluation and Research has informed Genzyme that there are no outstanding issues relating to the preclinical data for Fabrazyme.

2.1 Summary of Preclinical Pharmacology and Toxicology Studies

Several preclinical studies were conducted to evaluate the pharmacologic and toxicologic potential of Fabrazyme.

The studies included single dose and/or repeated dose studies in dogs, primates and rodents including the α GAL SV129 Knock-out mouse.

2.1.1 Pharmacodynamics

Pharmacodynamic studies were conducted using α GAL Knock-out SV129 mice to investigate the effect of Fabrazyme on GL-3 in a variety of tissues. Key findings from these studies showed:

- IV administration of Fabrazyme at 0.03, 0.1, 0.3 and 3.0 mg/kg, including the dose level to be used clinically (1.0 mg/kg), significantly reduced GL-3 levels at all doses tested in a time and dose dependent manner;
- reductions were observed in the liver, heart, spleen and kidney;
- cumulative doses of 0.5-0.6 mg/kg Fabrazyme cleared excess GL-3 in the liver after 1-2 days, whereas cumulative doses of 5-6 mg/kg were required for complete reductions in the kidney, heart and spleen;
- following a single dose of 3.0 mg/kg, GL-3 levels in the kidney decreased by 24% at Week 1 and gradual reaccumulation was observed thereafter to control levels at Week 6; and

- no undesirable pharmacodynamic effects were identified.

Biodistribution studies following a single IV administration demonstrated:

- in mice and rats given single intravenous bolus dose of 0.25-3.0 mg/kg, most of the Fabrazyme activity was present in the liver (22-38% of injected dose) followed by spleen, and kidneys (1.6-11% of injected dose) with little to none recovered in the lungs, heart, and brain. There was no difference in the biodistribution of Fabrazyme in the rat when 3.0 mg/kg dose was administered either as a bolus or as 2 hour infusion; hence, preclinical studies using an IV bolus are appropriate and relevant in support of clinical studies using an IV infusion.

Studies were conducted to evaluate the potential for Fabrazyme to accumulate in the liver following repeated intravenous administration.

- In the rat study, accumulation of Fabrazyme in rat livers 24 hours after the last of 27 weekly doses was noted at the 30 mg/kg dose. No histopathologic or liver enzyme changes were associated with this accumulation.
- The amount of Fabrazyme recovered from monkey liver tissue one day after every other week administration for 26 weeks of Fabrazyme at 3, 12, or 48 mg/kg was proportional to the dose administered. The data indicate that there is no accumulation of Fabrazyme in monkey livers with this dosing regimen.

A study was conducted to evaluate the effects of a single bolus IV administration of Fabrazyme on cardiac function in Beagle dogs.

- Doses used in this study were 0, 3, 9, and 27 mg/kg. Administration of escalating doses of Fabrazyme to Beagle dogs showed no adverse cardiac effects at doses of 3 and 9 mg/kg. A transient hypotension (decrease from 30 – 40 mmHg that returned to normal within 40 minutes of dosing) was observed in 5 of 6 dogs administered Fabrazyme at a dose of 27 mg/kg. Heart rate, respiration rate, and central venous pressure were not significantly affected.

2.1.2 Pharmacokinetics

Pharmacokinetics of IV infusion of Fabrazyme were evaluated in the rodent, dog and the monkey.

- Pharmacokinetics were linear at low doses of (3 mg/kg) and non-linear at higher doses (9 and 27 mg/kg) in rats and dogs. Fabrazyme was cleared with first order kinetics at low dosages, whereas at higher dosages, initial clearance was relatively slow. In monkeys, following a six-hour intravenous infusion of Fabrazyme every other week for 25 weeks at 3, 12, or 48 mg/kg, the pharmacokinetics were linear at all doses tested. This was likely due to the slow infusion rather than bolus administration.
- There were no differences noted in the pharmacokinetic parameters between the sexes in any studies.

2.1.3 Toxicology

Nonclinical data reveal no special hazard for humans based on studies to evaluate single dose toxicity and repeated dose toxicity. Genotoxic and carcinogenic potential are not expected.

- Two single-dose toxicology studies were carried out in Sprague-Dawley rats. Doses up to 27 mg/kg were administered by bolus injection. No toxic effects were observed. The dose of up to 27 mg/kg is 9 times the highest dose for the Phase 1/2 Study and 27 times higher than the dose used in the Phase 3 Placebo-Controlled Study and the Phase 2 Open-Label Study (Japan) and the proposed dose for labeling.
- Two repeat dose studies were carried out in Sprague-Dawley rats and cynomolgus monkeys. In the rat repeat-dose study, animals received a weekly injection of up to 30 mg/kg Fabrazyme for 27 weeks. In addition, two rats of each sex in each of the high and control dose groups were left untreated for a further four weeks after the end of the 27 week dosing period. No adverse signs of toxicity were observed. However, after the third week of dosing of Fabrazyme (Week 3) some rats showed extreme hypoactivity associated with hypersensitivity requiring treatment with diphenhydramine (DPH). This was not unexpected considering that a human protein was administered to rats in this study. Pre-treatment with DPH at 5 mg/kg was successful in blocking this response. In the repeat-dose monkey study, Fabrazyme was administered by IV infusion every other week for 25 weeks at doses of 3, 12, and 48 mg/kg. Fabrazyme was well tolerated and minimal clinical observations were noted in the 12 and 48 mg/kg dosing groups that were likely associated with a mild hypersensitivity response in some animals. This response was not serious enough to require treatment.
- Based on the available toxicology data, Fabrazyme is deemed safe at up to 48 times the recommended human dose.
- On a mg/mg basis the available toxicology data support the safety of Fabrazyme at 48 times the recommended human dose.

2.1.4 Reproduction Studies

A study to evaluate the effects of Fabrazyme on embryo-fetal development (Segment II) was performed in rats at doses of 3, 10 and 30 mg/kg/day from gestational Day 7-17. Hepatocellular necrosis consistent with accumulation of test material when administered at high doses daily was evident in maternal livers upon histological evaluation. There were no effects of Fabrazyme on embryo-fetal development. However, it is proposed to include statements in the labeling that Fabrazyme should only be used in pregnant or lactating women if clinically justified.

2.1.5 Mutagenicity/Carcinogenicity

There have been no studies conducted to assess the mutagenicity and/or carcinogenicity of Fabrazyme. The structure of the drug substance (a glycoprotein), its purity profile and the excipients of the final product (mannitol and sodium phosphate) do not suggest that the material has any mutagenic or carcinogenic potential. In addition the range and type of genotoxicity

studies routinely conducted for pharmaceuticals are generally not applicable to biotechnology-derived pharmaceuticals and therefore not performed (ICH S6 Guideline).

2.1.6 Dosage Regimen

Maximum clearance of GL-3 was seen 1-2 weeks after a single administration of Fabrazyme at the recommended clinical dose (1.0 mg/kg) and re-accumulation did not occur for approximately one month post-dose in the liver, heart, and spleen but gradual reaccumulation was evident in the kidney at week 2. In addition, tissue half life of Fabrazyme varied from 1-7 days in different tissues. Both findings support a clinical administration regimen of an approximate dosing interval of 14 days.

2.1.7 Conclusions

In conclusion, the preclinical data clearly support clinical use of Fabrazyme at the proposed dose of 1.0 mg/kg, every two weeks.

3. FABRAZYME® CLINICAL DEVELOPMENT PROGRAM

The clinical development of Fabrazyme has included several studies conducted on a worldwide population of patients with Fabry disease. (Table 3-1).

Table 3-1 Summary of Clinical Studies with Fabrazyme

Protocol No. and Study Design	Primary Endpoint(s)	Fabrazyme Treatment Doses	Duration of Treatment	No. Patients Entered/ Evaluable
FB9702-01 Phase 1/2 Study Open-label, Non-randomized, Dose-finding	Clinical pharmaco-dynamic, pharmacokinetic, and safety parameters.	0.3 mg/kg q14d X 5 1.0 mg/kg q14d X 5 3.0 mg/kg q14d X 5 1.0 mg/kg q48 hrs X 5 3.0 mg/kg q 48 hrs X 5	10 Weeks " " 10 Days "	15/15 (15 male) (n=3/dose group)
AGAL-1-002-98 Phase 3 Double-Blind Placebo-Controlled Study Randomized, Double-blind, Placebo-Controlled	Morphological assessment of GL-3 inclusions of the capillary endothelium of the kidney.	Fabrazyme: ~1.0 mg/kg q14d X 11	20 weeks	29/29 (27 male, 2 female)
		Placebo: ~1.0 mg/kg q14d X 11	20 weeks	29/29 (29 male)
AGAL-006-99 Phase 1/2 Extension Study	Ongoing assessments of safety and efficacy parameters of the Phase 1/2 Open-label study.	~1.0 mg/kg q14d	Until marketing approval	13/13 (13 male) Ongoing
AGAL-005-99 Phase 3 Extension Study Open-label extension study (patients rolled over from Study AGAL-1-002-98)	Ongoing assessments of safety and efficacy parameters of the Phase 3 Placebo-Controlled Study (AGAL-1-002-98)	~1.0 mg/kg q14d	approx. 5 years	58/58 (56 male, 2 female) Ongoing
AGAL-007-99 Phase 2 Open-Label Study Non-randomized, bridging to AGAL-1-002-98	Morphological assessment of GL-3 inclusions of the capillary endothelium of the kidney.	~1.0 mg/kg q14d X 11	20 weeks	13/13 (13 male)
AGAL-008-00 Multi-center, double-blind, randomized, placebo-controlled Phase 4 study	Time to clinically significant progression of the composite outcomes of renal, cardiac, and cerebral vascular disease, and/or death among Fabry patients with advanced disease.	Fabrazyme: ~1.0 mg/kg q14d X 11	~35 months	75; (Blinded) (Randomization is 2:1, active: placebo) Ongoing
		Placebo: ~1.0 mg/kg q14d X 11		
AGAL-012-01 Multi-center, open-label extension study (patients rolled over from Study AGAL-007-99)	Ongoing assessments of safety and efficacy of the Open-label, Non-randomized study	~1.0 mg/kg q14d	Until marketing approval	13/13 (13 male); Ongoing
AGAL-019-01 Multi-center, open-label safety study	Evaluation of safety of re-challenging Fabry patients who have previously tested IgE positive or have had positive skin test to r-hαGAL	0.5 mg/kg q7d x 2 Followed by 1.0 mg/kg q14d	Up to 52 weeks	3 infused; Ongoing

3.1 Clinical Pharmacology

A Phase 1/2 Study in Fabry patients which investigated safety, pharmacokinetic and pharmacodynamic measurements, and dose-response effects was conducted in 15 male patients. The study evaluated the effects of 5 different intravenous dosing regimens with three patients in each regimen:

- 0.3 mg/kg q 14 days for 5 doses;
- 1.0 mg/kg q 14 days for 5 doses;
- 3.0 mg/kg q 14 days for 5 doses;
- 1.0 mg/kg q 48hrs for 5 doses; and
- 3.0 mg/kg q 48hrs for 5 doses.

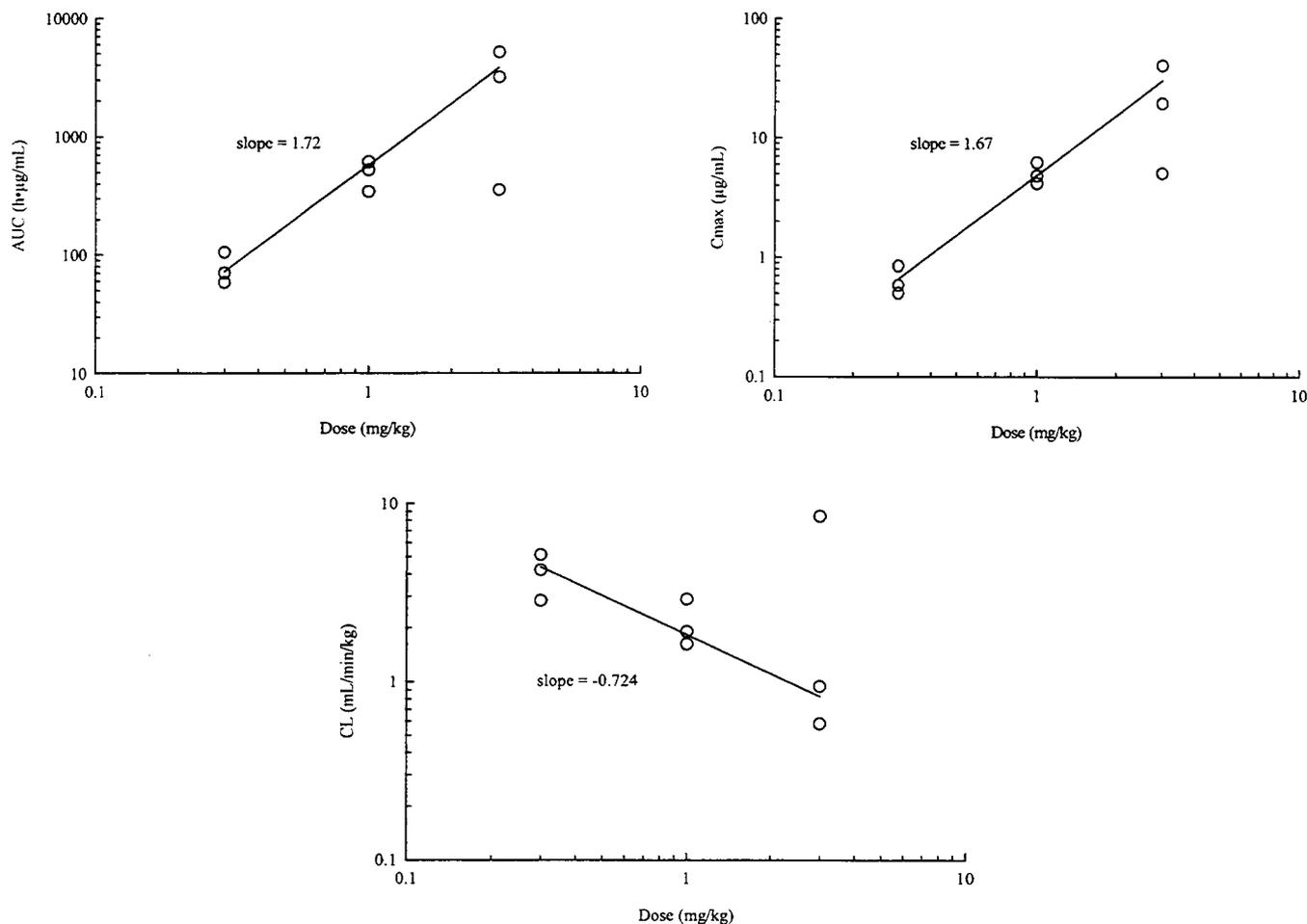
In addition, the pharmacokinetics of Fabrazyme were evaluated in 11 European patients with Fabry disease who participated in the Phase 3 Placebo-Controlled Study.

Results of the Phase 1/2 Study were published in Issue 3, 2001 of the American Journal of Human Genetics (*Eng, 2001, Am J Hum Genet*) (Appendix 8.2).

3.1.1 Pharmacokinetics Results of the Phase 1/2 Study and Phase 3 Placebo-Controlled Study

Plasma profiles of Fabrazyme were studied at 0.3, 1.0 and 3.0 mg/kg in the Phase 1/2 Study. The area under the plasma concentration-time curve (AUC_{∞}) and the clearance did not increase proportionately with increasing doses, demonstrating that the enzyme follows non-linear pharmacokinetics. Terminal half-life was dose independent with a range of 45 - 102 minutes. Figure 3-1 illustrates the relationship between AUC_{∞} , C_{max} , CL and dose after Infusion 1 of Fabrazyme.

Figure 3-1 Relationship between AUC_{∞} , C_{max} , CL and Dose after Infusion 1 of Fabrazyme (FB9702-01)



Pharmacokinetics of Fabrazyme was evaluated in 11 Fabry patients in Europe participating in the Phase 3 Placebo-Controlled Study. Table 3-2 summarizes the ranges of pharmacokinetic parameters following an intravenous infusion of 1.0 mg/kg of Fabrazyme over a period averaging 280 to 300 minutes in the Phase 3 Placebo-Controlled Study. There was no correlation of IgG seroconversion and IgG titer to AUC and half-life.

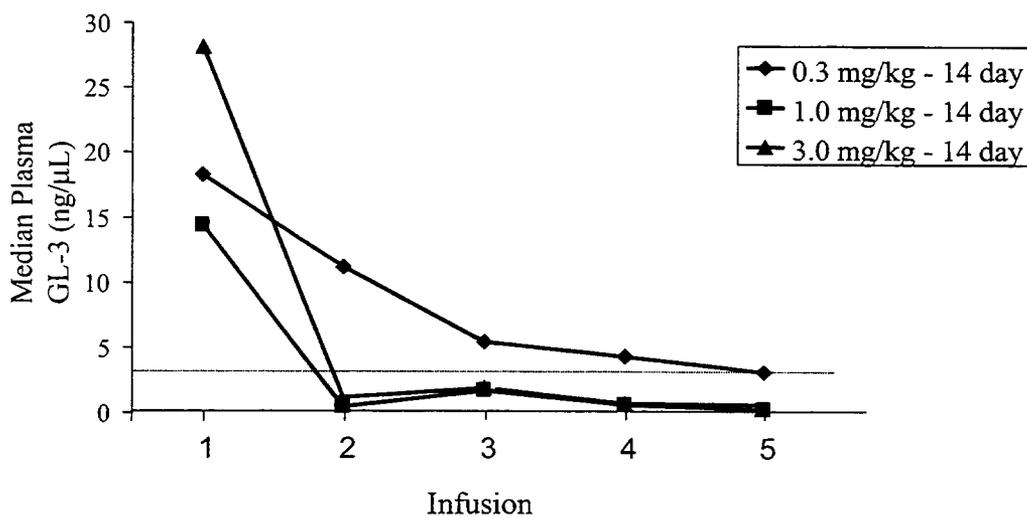
Table 3-2 Pharmacokinetic Parameters After Infusion of Fabrazyme in the Phase 3 Placebo-Controlled Study

Pharmacokinetic Parameter	Range of Means
Maximum Plasma Concentration (C_{max})	2.09 – 3.49 $\mu\text{g/mL}$
Area Under the Curve (AUC_{∞})	372 – 784 $\mu\text{g/mL}\cdot\text{min}$
Volume of Distribution (V_z)	0.23 – 0.49 L/kg
Volume of Distribution at Steady State (V_{ss})	0.12 – 0.57 L/kg
Plasma Clearance (CL)	1.75 – 4.87 ml/min/kg
Half-Life ($t_{1/2}$)	82.3 – 119 minutes

Pharmacokinetics of Fabrazyme were also evaluated in 13 Fabry patients in Japan participating in a Phase 2 Open-Label Study (Japan). The results of these evaluations show that Fabrazyme pharmacokinetics are comparable in Caucasian and Japanese Fabry patients.

3.1.2 Pharmacodynamics and Dose Response

The Phase 1/2 Study demonstrated that Fabrazyme was taken up by cells and was effective in clearing GL-3 from multiple cell types but most importantly it effectively cleared GL-3 from vascular endothelial cells to normal or near normal levels. When taken together, the data from histologic evaluations and biochemical analyses of GL-3 reduction in tissue and plasma suggest a discernible dose response in clearing GL-3 stores, with higher doses being more effective than lower doses. This response was most evident in plasma GL-3 levels. In the 14-day schedule, both the 1 and 3 mg/kg dosing groups responded by lowering GL-3 to minimal levels by the second infusion, whereas for the 0.3 mg/kg group, although a decrease in plasma GL-3 levels was noted, it was not to the same degree. (Figure 3-2)

Figure 3-2 Effect of Fabrazyme Dose on Plasma GL-3 Clearance

3.2 Clinical Efficacy – Phase 3 Placebo-Controlled Study

3.2.1 Selection of Treatment Dose and Regimen

Following infusion, Fabrazyme is taken up from the plasma at least in part by way of glycoreceptors in cells such as endothelial cells, leukocytes, fibroblasts, mesangial cells, cardiomyocytes, and/or podocytes. The pharmacodynamic activity of Fabrazyme occurs in the lysosomes of these cells.

Human pharmacokinetic findings were consistent with the animal model studies. The clearance of Fabrazyme in rats and dogs was relatively slow at higher doses until serum concentrations decreased to below a threshold level, whereupon more rapid clearance followed. The non-linear nature of the disposition observed in humans and animals is consistent with a saturable elimination mechanism in accordance with the general understanding of the fate of glycoproteins. The pharmacokinetics of Fabrazyme appeared unaltered following repeated administration.

The dose interval of every two weeks is based on the animal and human pharmacokinetics data supported by the findings in studies of knock-out mice in which GL-3 reduction in target tissues persisted for several weeks post-Fabrazyme administration.

In consideration of the preclinical results, and clinical safety and preliminary efficacy results observed in the Phase 1/2 Study, the mid-dose of 1.0 mg/kg every 14 days offered the optimal balance between GL-3 clearance and frequency of infusion associated reactions. Therefore, the 1.0 mg/kg every 14 days was selected for future clinical studies. In the Phase 3 Placebo-Controlled Study, a range of 0.9 – 1.0 mg/kg was permitted in order to accommodate patient weight, to permit flexibility in treatment vial preparation, and to maximize product use among the available vials.

In addition, in comparison to the Phase 1/2 Study, a slower infusion rate and a higher dilution volume were selected for the Phase 3 Placebo-Controlled Study. A volume of 500 mL was selected to be infused at a rate of 0.25 mg/min, a duration of approximately 6 hours. This rate was selected in order to reduce the potential for infusion associated events that could compromise the blinding scheme of the Phase 3 Placebo-Controlled Study for the patients and investigators.

3.2.2 Selection of Endpoints

3.2.2.1 Considerations in Selection of Appropriate Study Endpoints

In designing a Phase 3 Placebo-Controlled Study to evaluate the therapeutic benefit of Fabrazyme for treatment of Fabry disease, a careful consideration of the clinical manifestations of Fabry disease is essential. The major clinical manifestations include pain, renal insufficiency,

stroke and transient ischemic attacks, and myocardial disease. Each of these was given careful consideration as a possible primary endpoint to assess the efficacy of therapy with Fabrazyme.

Kidney failure is a major cause of morbidity and mortality in Fabry patients. Based on the pathophysiology of the renal disease, it was concluded that a better clinical endpoint would be to focus on preserving renal function, thereby avoiding the most devastating common complication of the disease. The likelihood that one could improve renal function once glomerulosclerosis had occurred was felt to be unlikely.

- Since renal insufficiency progresses over the course of years, it was concluded that to properly design a study using preservation of renal function as the primary endpoint, particularly among patients with a normal renal function (as measured by serum creatinine or GFR), would require a relatively large number of patients studied over several years, as in studies of patients with diabetes. This was not felt to be feasible in an ultra-orphan population.
- Careful consideration was also given to designing a study focusing solely on the subset of Fabry patients who already have abnormal renal function based on increased serum creatinine and decreased GFR. However, our analysis revealed that even focusing on this narrow subset of Fabry patients would require a study of approximately three years' duration. Such a study has been incorporated as an important part of our Phase 4 Program (Section 6)

While **pain** is often a presenting symptom of the disease and improvement in pain was ultimately incorporated as a secondary endpoint, it was not chosen as the primary endpoint for several reasons.

- Pain is subjective in nature and highly variable in extent, making it challenging to measure quantitatively.
- No pain instruments are validated for assessing pain related to Fabry disease.
- Fabry pain can often be well controlled using currently available pain medications; however, use of pain medications can confound interpretation of results and, therefore, their usage must be recorded accurately (*Agency for Healthcare Research and Quality, 2002*).
- Pain improvement as the primary endpoint required statistically powering the study with more than 100 Fabry patients who had significant pain on pain medications in consideration of the placebo effect that is often observed in a study setting and based upon other well-controlled pain studies for other indications. This was not feasible given the very small size of this patient population.
- False positive and/or false negative pain findings are common in small studies (*Moore, 1998, Pain*).
- Fabry pain often wanes over time and it would be very difficult, if not impossible, to account for this in the sizing of such a study in this ultra-orphan disease population.

- In small studies, pain assay sensitivity may be too low to distinguish between active and inactive treatment, where assay activity is defined as the ability of a study to distinguish between active and inactive treatments; (*Temple, 2000, Ann Intern Med*).
- Pain results, as a subjective endpoint, could be potentially biased even with mild infusion reactions that might lead to breaking of the blind.

Prevention of clinically significant **cardiac** or **cerebrovascular** events were also considered as primary endpoints. However, these events:

- Are episodic in nature with an event rate so poorly documented that determination of sample size and study duration were not feasible;
- Are confounded by common concomitant conditions such as hypercholesterolemia and hypertension; and
- Frequently represent a final isolated vascular occlusion. Therefore, patients may already have significant occult vascular disease and may have such an increased risk for vascular events that treatment at this late-stage with Fabrazyme will not be very effective.

3.2.2.2 Selection of the Primary Endpoint: GL-3 Clearance

Because of the feasibility concerns outlined above surrounding conducting a properly powered pivotal study using a clinical endpoint in this ultra orphan disease, and after extensive discussions between Genzyme, FDA and experts in the field, a surrogate endpoint likely to predict clinical benefit was mutually identified and agreed upon with FDA as being appropriate for studying as the primary endpoint in the Phase 3 Placebo-Controlled Study. Since kidney failure is a major cause of morbidity and mortality in Fabry patients, a surrogate endpoint for the progression of renal disease was considered further.

The mutually agreed upon primary endpoint was the histologic clearance to normal or near normal levels (i.e., 0 score on a scale of 0-3) of GL-3 accumulations from the interstitial capillary endothelial cells of the kidney.

The rationale for agreeing to this primary endpoint was that:

- Renal failure is the most common devastating feature of Fabry disease;
- Glomerulosclerosis present in Fabry disease is primarily a result of vascular damage;
- Vascular endothelial GL-3 deposition is the pathologic basis of morbidity and mortality
 - Supported by pathology in cardiac variant patients (Section 1.4)
 - Supported by pathology in female heterozygotes (Section 1.4);
- This endpoint could be measured in a reasonable timeframe;
- Statistical power calculations based on this endpoint showed that the sample size was appropriate for this rare disease; and

- Clearance of GL-3 to normal or near-normal levels was felt to be clinically important based on discussions between Genzyme, FDA, and outside experts and was, therefore, expected to predict normal function and clinical benefit.
- For insidious diseases, such as kidney failure, that lack overt clinical symptoms until they are beyond the reach of medical therapy, histologic diagnosis often can be the only objective indicator for staging disease severity and gauging the success of therapy.

The decision to focus the primary endpoint specifically on clearance of abnormal GL-3 accumulations in *interstitial* capillary endothelial cells as opposed to glomerular endothelial cells or as opposed to changes in the degree and extent of glomerulosclerosis was based upon several important practical concerns. Typical kidney biopsies contain only a small number of glomeruli. In fact, not infrequently, no glomeruli are present. Hence, if one focused primarily on glomerular capillary endothelial cells, some biopsies would be completely uninformative and others would have so few cells present that sampling variability might mislead one. Similarly, if one tried to assess the extent of focal segmental glomerulosclerosis and global glomerulosclerosis in each biopsy, the small number of glomeruli in each sample makes the assessment highly subject to sampling variability. Additionally, glomerulosclerosis is generally believed to be irreversible and it would be unreasonable to expect to observe reversal of sclerosis. For these reasons, we do not believe that changes in extracellular mesangial matrix and glomerulosclerosis are the best indicators of renal health or disease in Fabry patients. We have therefore assessed GL-3 clearance within multiple cell types in the kidney with the primary endpoint being clearance of abnormal GL-3 accumulations in *interstitial* capillary endothelial cells. A minimum of 50 endothelial cells were assessed in each biopsy.

3.2.3 Study Design

The pivotal Phase 3 Placebo-Controlled Study was a multinational (4 countries), multicenter (eight study sites), placebo-controlled, double blind, randomized study of patients diagnosed with Fabry disease having had no prior treatment with enzyme replacement therapy. A randomized, double-blind study design was employed to minimize the potential for subjective bias. Overall, the study design attempted to capture and isolate from placebo the effects of enzyme replacement therapy with Fabrazyme in Fabry disease patients.

Fifty-eight patients were randomized to one of two treatment arms (active treatment or placebo) at eight study centers. Baseline assessments for all safety and efficacy endpoints and procedures were completed within 28 days prior to Visit 1 (first infusion visit). Patients received approximately 1.0 mg/kg (0.9 to 1.1 mg/kg) of Fabrazyme or placebo intravenously at a rate of

no more than 0.25 mg/min over a period of approximately 6 hours every 2 weeks, for a total of 11 infusions. Patients were observed in the clinic for 2 hours after each infusion.

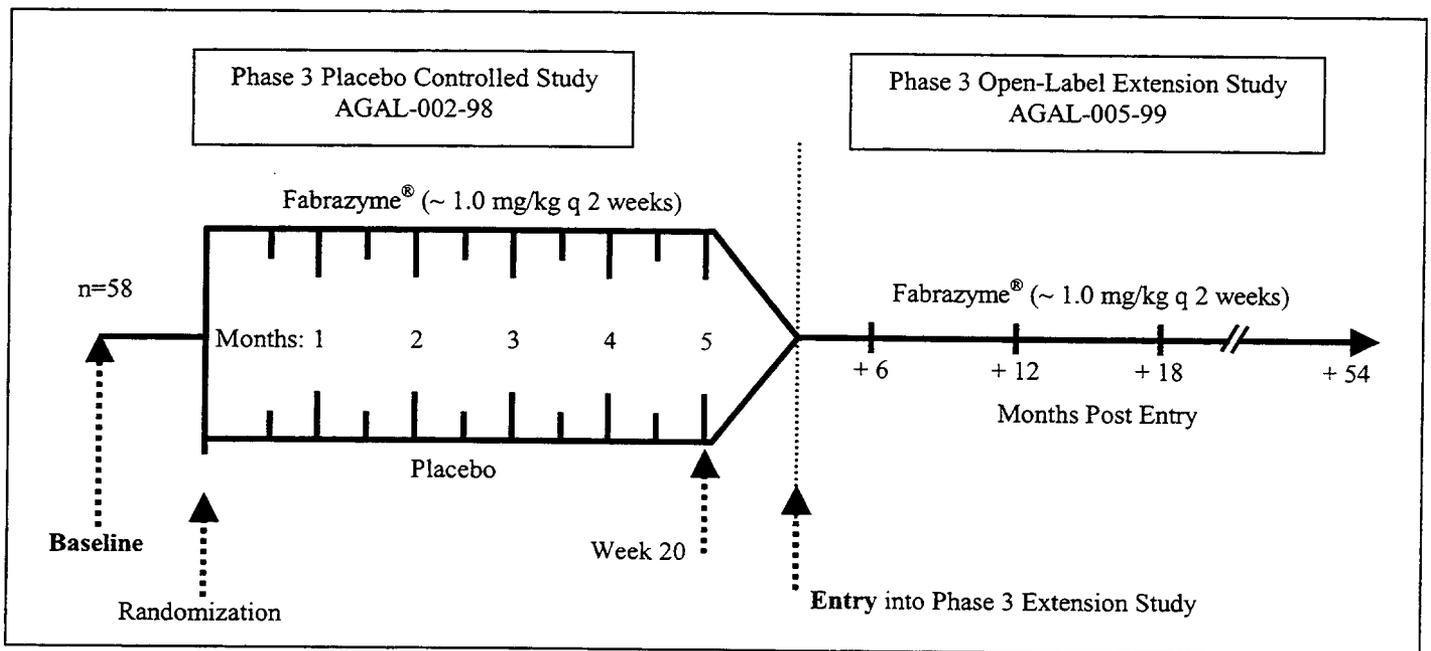
Patients who successfully completed the Phase 3 Placebo-Controlled Study were eligible for entry into a Phase 3 Extension Study. All 58 patients completed the Phase 3 Placebo-Controlled Study and elected to enroll in the Phase 3 Extension Study. During this ongoing extension study, all patients are receiving Fabrazyme, and long-term safety and efficacy parameters are being monitored. This study will continue assessments for an additional 4.5 years, for a total of up to five years of patient treatment with Fabrazyme. Important features of the extension study include:

- The opportunity to confirm the results of the Phase 3 Placebo-Controlled Study by the crossover of placebo patients to Fabrazyme treatment;
- The opportunity to collect additional long-term safety and efficacy information specifically with regard to the surrogate endpoints and clinical outcome evaluations;
- The opportunity to determine whether patients can tolerate an increased infusion rate; and
- The transfer of patients to local treatment sites for infusions and study assessments, providing patients with easier access to the study drug treatment.

Currently, this study is being conducted at 19 investigational sites in the U.S. and Europe.

A schematic outline of the Phase 3 Placebo-Controlled Study and Phase 3 Extension Study is presented in Figure 3-3.

Figure 3-3 Study Outline



3.2.3.1 Estimation of Sample Size

Table 3-3 displays the estimated power calculations (as described in the protocol) given the possible success rates within each treatment group. These estimates were calculated for a two-tailed chi-square test with continuity correction. The α was set at 0.050 and the estimated number of patients per group was 28 (overall total = 56).

Table 3-3 Estimated Power Calculations

Number (%) Patients Scored 0 in the Control Group	Number (%) of Patients Score 0 in the Fabrazyme Group		
	10 (35.7)	11 (39.2)	12 (42.9)
0 (0)	90%	94%	97%
1 (3.6)	78%	85%	91%
2 (7.1)	63%	73%	81%
3 (10.7)	47%	58%	68%

3.2.3.2 Evaluation of the Primary Endpoint and other Histologic Endpoints

The primary endpoint of the Phase 3 Placebo Controlled Study was the clearance of GL-3 accumulation from the interstitial capillary endothelial cells of the kidney to normal or near-normal levels.

In addition to this primary endpoint, the interstitial capillary endothelial cells of the skin and heart were evaluated.

Kidney, skin, and heart biopsies were performed during the Baseline period and again at the final study visit. After the samples were processed, under blinded conditions, groups of three independent renal, cardiac, and skin pathologists used light microscopy (LM) to score the severity of GL-3 accumulation in the capillary endothelium of each of the samples using the following scoring system:

- **None:** Score = 0 – A zero score is confined to vascular endothelium in which there is either no evidence of visible endothelial inclusions in the vessels examined, or, in which the majority of the vessels contain no visible inclusion and the remaining vessels contain only traces of visible inclusions. A trace is defined as vessels containing only single, isolated, small inclusions in the remaining minority of the vessels examined. Scores will reflect the pathologists' overall impression of the patients' endothelial status, ignoring clearly aberrant vessels or artifacts.
- **Mild:** Score = 1 – The criteria for designation of mild is that the majority of vessels in the fields examined contain evidence of inclusions in the cytoplasm of the endothelium. This grade ranges from single small granules in almost all vessels to occasional multiple loosely organized granules often distributed randomly in different areas of the cytoplasm. Occasional vessels may have small clusters of granules. There is rarely evidence of bulging into the capillary lumen. Scores will reflect the pathologists' overall impression of the patients' endothelial status, ignoring clearly aberrant vessels or artifacts.
- **Moderate:** Score = 2 – A score of moderate is characterized by multiple areas throughout the endothelial cytoplasm containing single or multiple granules often in small clusters. All or nearly all clearly defined capillaries will contain multiple inclusions. In the juxtannuclear area there may be occasional coalescing of inclusions with minor bulging into the vessel lumen. Coalescence is not a feature away from the nucleus.

Scores will reflect the pathologists' overall impression of the patients' endothelial status, ignoring clearly aberrant vessels or artifacts.

- **Severe:** Score = 3 – A score of severe is reserved for vascular endothelium containing large accumulations of inclusions, some of which coalesce into clusters at both the juxtannuclear region and around the free cytoplasmic borders, and where many bulge into the vessel lumen. In the severe category the majority of vessels are readily identified as containing large numerous granules often at several different sites around the periphery of the endothelium. Scores will reflect the pathologists' overall impression of the patients' endothelial status, ignoring clearly aberrant vessels or artifacts.

Subsequent to the initial histological review of the capillary endothelium of the tissue samples, but prior to unblinding, a second review of the kidney tissue samples alone, the primary study endpoint, was prompted by discussions between the FDA and Genzyme. These discussions led to a mutual agreement of a more quantitative scoring system to review the light microscopy slides of the kidney biopsy specimen. The slides and photomicrographs to be reviewed in this second analysis were restricted to those samples that had been scored as a "0" or "1" during the first evaluation. The same LM slide for each patient that had been evaluated by the pathologists during their first review was to be used for their second review. This quantitative assessment was used to define the primary endpoint and is described below and displayed in Figure 3-4:

1. A minimum of 50 vessels was to be assessed. Each assessable vessel in a slide was read by the pathologists and classified as follows:
 - Number of Clear Vessels [0]: an interstitial capillary that contains no visible inclusions in the endothelium as judged by LM using a total magnification of 1000X in conjunction with oil immersion.
 - Number of Vessels scored as Trace [trace]: an interstitial capillary that contains a single, small (approximately 0.2 (\pm 0.1) microns) lipid granule within the endothelium as judged by LM using a total magnification of 1000X in conjunction with oil immersion. For capillaries obviously cut in the longitudinal plane such that the length of the visible lumen is greater than twice the width, two small (approximately 0.2 (\pm 0.1) microns) inclusions will be accepted.
 - Number of Vessels scored as Mild [1]: an interstitial capillary that contains multiple discrete lipid granules within the endothelium as judged by LM using a total magnification of 1000X in conjunction with oil immersion.
 - Number of Vessels scored as Moderate [2]: an interstitial capillary that contains a single or multiple aggregates of lipid granules within the endothelium as judged by LM using a total magnification of 1000X in conjunction with oil immersion.
 - Number of Vessels scored as Severe [3]: an interstitial capillary that contains aggregates of lipid granules within the endothelium that are neither large enough or numerous enough to cause clear distortion of the luminal surface of the endothelial cell as judged by LM using a total magnification of 1000X in conjunction with oil immersion.
2. If the majority of vessels were scored as None (0) and less than 5% of vessels were scored as \geq 1, then the slide was given a new Majority Score of 0. Otherwise, the slide was given a new Majority Score of 1.
3. The new Majority Score supersedes the previous original Majority Score.

Kidney, skin, and heart biopsies were conducted at specific timepoints during the Phase 3 Placebo-Controlled Study and the Phase 3 Extension Study. The number of patients

(denominator) may change at each timepoint because biopsies may not have been conducted or may have not been evaluable. (Table 3-4)

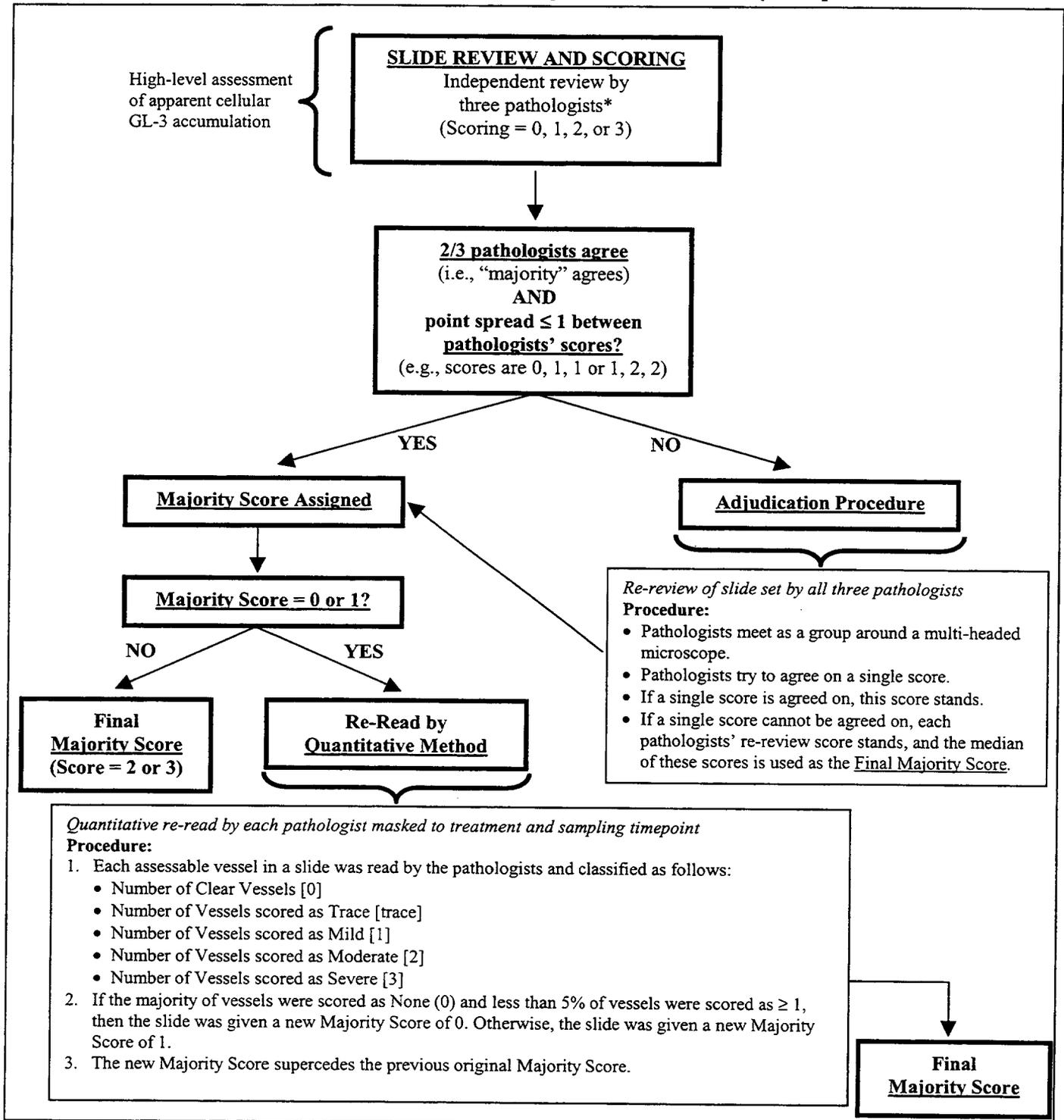
Table 3-4 Number of Evaluable Tissue Biopsies Obtained at Different Study Timepoints

Biopsied Tissue	Phase 3 Placebo-Controlled Study				Phase 3 Extension Study					
	Baseline		Week 20		6 Months		12 Months		18 Months	
	PL	FZ	PL	FZ	PL/FZ	FZ/FZ	PL/FZ	FZ/FZ	PL/FZ	FZ/FZ
Kidney	29	29	29	28	24*	25*	-	-	-	-
Skin	29	29	29	29	26	27	27	28	22*	24*
Heart	29	29	29	29	18*	22*	-	-	-	-

* Kidney and heart biopsies at the 6-month timepoint of the Phase 3 Extension Study may have been postponed to one year. Skin biopsies at the 18-month timepoint were optional.
PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

A secondary (histologic) endpoint was the mean between-group change in the combined GL-3 accumulation score in the capillary endothelium of the kidney, heart, and skin from Baseline to Week 20 using light microscopy. Interstitial capillary endothelial cells from each organ were scored on a scale of 0 – 3.

Figure 3-4 Slide Review and Scoring Diagram for the Primary Endpoint



* Renal pathologists were:

Dr. Helmut G. Rennke, M.D.: Professor of Pathology, Harvard Medical School; Director of Renal Pathology Laboratory, Brigham and Women’s Hospital;

Robert B. Colvin, M.D.: Benjamin Castleman Professor of Pathology, Harvard Medical School; Chief, Department of Pathology, Massachusetts General Hospital; and

Steven Dikman, M.D.: Associate Professor of Renal Pathology; Mount Sinai School of Medicine New York, NY

3.2.3.3 Secondary, Tertiary, and Other Endpoints

In addition to the secondary histologic endpoint described above, two additional secondary endpoints were chosen to demonstrate the efficacy of Fabrazyme compared to placebo:

- Change in GL-3 levels from Baseline to Week 20 measured by a rank sum score of kidney tissue and urine levels as measured by an ELISA assay, and
- Reduction of pain from Baseline to Week 20 as assessed by the Short Form McGill Pain Questionnaire

The tertiary endpoints of the Phase 3 Placebo-Controlled Study included the determination of change from Baseline to Week 20 in:

- Vibration Detection Threshold;
- Neuropathy Impairment Score;
- SF-36 Health Survey Scores;
- Total Symptom Score (the neuropathic signs and symptoms of pain, burning, paraesthesia, and numbness, are quantified by this scoring system);
- Physician Assessment of Fabry Symptoms;
- Pain as assessed by a Daily Patient Diary;
- GFR as measured by inulin clearance or creatinine clearance tests, and
- Autonomic status as measured by the composite score of the Quantitative Sudomotor Axon Reflex Test (QSART), Thermal/Vibration Detection Threshold Assessment, and Venous Occlusion Plethysmography.

The other endpoints of the Phase 3 Placebo-Controlled Study included determination of change from Baseline to Week 20 in:

- Serum Creatinine
- Urinary Protein:Creatinine Ratio
- Plasma GL-3
- Ophthalmic Examination

3.2.3.4 Additional Histologic Analyses

After the Phase 3 Placebo-Controlled Study was completed, in response to questions asked by FDA relating to whether the reduction in GL-3 was isolated to the interstitial capillary endothelial cells, Genzyme undertook a retrospective histologic review of additional cell-types of the kidney and skin. (Section 4.2.1.2) These additional cell-types were also reviewed in the biopsy samples taken during the Phase 3 Extension Study and the Phase 2 Open-Label Study (Japan).

The additional **kidney** cell-types analyzed included:

- Glomerular endothelial cells
- Non-capillary (arteriolar) interstitial smooth muscle cells
- Non-capillary (arteriolar) interstitial endothelial cells
- Podocytes
- Distal convoluted tubules/collecting ducts
- Mesangial cells
- Interstitial cells

These additional kidney cell-types were analyzed in biopsies from three timepoints to demonstrate the long-term treatment effect: Baseline and Week 20 (Final Visit) of the Phase 3 Placebo-Controlled Study, and at 6 months into the Phase 3 Extension Study. In addition, these biopsies were also evaluated for changes in mesangial cell matrix and the number of glomeruli with focal segmental or global glomerulosclerosis.

The additional **skin** cell-types for analysis included:

- Deep vessel endothelial cells
- Deep vessel smooth muscle cells
- Cells of the perineurium

Biopsies were conducted at five timepoints to assess skin cell-types: Baseline and Week 20 (Final Visit) of the Phase 3 Placebo-Controlled Study, and at 6, 12, and 18 months into the Phase 3 Extension Study.

To evaluate these additional kidney and skin cell-types, additional refined scoring methods were devised that would permit a consistent approach to scoring the different cell-types based on cell morphology and physiology. Table 3-5 summarizes the scoring system used for the additional kidney and skin cell-types. Results for the analyses of these additional kidney and skin cell-types are reported in Section 4.2.1.2.

Table 3-5 Additional Kidney Cell-Type/Tissue GL-3 Accumulation Scoring Scheme

Tissue	Cell-Type	Scoring System
Kidney	Glomerular endothelial cells Non-capillary (arteriolar) interstitial smooth muscle cells Non-capillary (arteriolar) interstitial endothelial cells Podocytes* Distal convoluted tubule/collecting ducts*	0 = None or Trace Accumulation 1 = Mild Accumulation 2 = Moderate Accumulation 3 = Severe Accumulation
	Mesangial cells Interstitial cells	0 = No lipid granules 1 = Minimal lipid granules 2 = Numerous lipid granules
Skin	Deep vessel endothelial cells Deep vessel smooth muscle cells Cells of the perineurium	0 = None or Trace Accumulation 1 = Mild Accumulation 2 = Moderate Accumulation 3 = Severe Accumulation

* In the Phase 3 Placebo-Controlled Study, for podocytes and distal convoluted tubule/collecting ducts, scoring was conducted using an increase, decrease, or no change assessment.

4. CLINICAL EFFICACY AND SAFETY

4.1 Phase 3 Placebo-Controlled Study

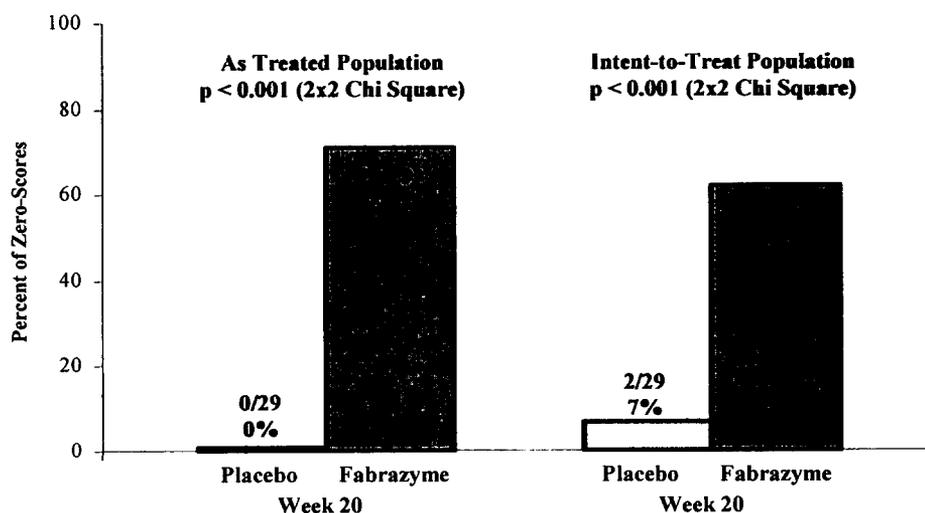
Results of the Phase 3 Double-Blind, Placebo-Controlled Study were published in the July 5, 2001 edition of the New England Journal of Medicine (*Eng, 2001, NEJM*) and results of further pathological findings were published in the November 18, 2002 edition of Kidney International (*Thurberg, 2002, Kidney Int*) (Appendix 8.2)

4.1.1 Primary Endpoint Results – Phase 3 Placebo-Controlled Study

The primary efficacy parameter for the Phase 3 Placebo-Controlled study was the between-treatment group histologic (using light microscopy) comparison of the clearance of GL-3 inclusions from the capillary endothelium of the kidney after 20 weeks of treatment with Fabrazyme. The primary endpoint analysis was based on the number of patients who had a majority score of zero at 20 weeks, signifying clearance of GL-3 to normal or near-normal levels in this cell-type.

Analyses were performed on the Intent-to-Treat population as well as the As Treated population. The As-Treated population takes into account treatment dispensing deviations that resulted in six patients receiving treatment not assigned by randomization. The statistical analyses performed on these two populations yielded similar results as shown in Figure 4-1 and Table 4-1 for the primary endpoint. For consistency, only the As Treated population is presented throughout this document for the remaining efficacy data. For all other efficacy parameters presented, the analysis performed on the Intent-to-Treat population yielded similar results to the As Treated population, with statistical significance being achieved in both populations for endpoints described in the protocol.

Figure 4-1 Kidney Biopsy LM Assessment: Between Treatment Group Comparison at Week 20 of Zero versus Non-Zero Using the Majority Score (As Treated and Intent-to-Treat Population)



There was a highly statistically significant difference ($p < 0.001$) between the Fabrazyme and placebo treatment groups. The greater proportion of patients with a kidney majority score of 0 occurred in the Fabrazyme group. In the As Treated population, 20/29 (69%) of Fabrazyme patients achieved the primary endpoint versus 0% in the placebo treatment group. The majority of patients who achieved a score of zero at Week 20 had scores at Baseline of moderate or severe (score of 2 or 3). Therefore, most patients demonstrated a two- or three-point reduction with Fabrazyme treatment. Eight out of the nine patients who did not achieve a zero-score had a score of one (mild) at Week 20. The remaining patient did not have a Week 20 biopsy and was assigned a score of 3 (severe). The odds of achieving a score of zero for patients treated with Fabrazyme compared to placebo is 99%. The results were consistent among the three, blinded renal pathologists who performed the assessments, across the study sites, and various subgroups.

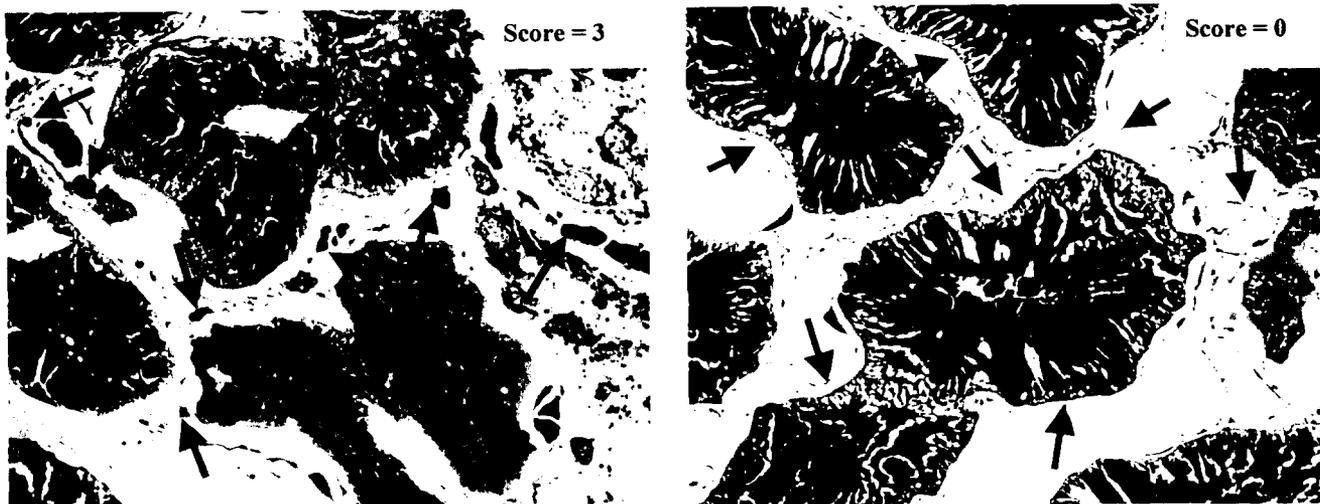
Table 4-1 Kidney Biopsy LM Assessment: Between Treatment Group Comparison at Week 20 of Zero versus Non-Zero Using the Majority Score (As Treated and Intent-to-Treat Populations)

Population	Treatment Group	Statistic	Tissue GL-3 Status			
			Zero	Non-Zero*	Odds Ratio (95% C.I.)	P-Value**
As Treated	Fabrazyme n = 29	n (%)	20 (69)	9 (31)	0.008 (0.00, 0.14)	<0.001
	Placebo n = 29	n (%)	0 (0)	29 (100)		
Intent-To-Treat	Fabrazyme n = 29	n (%)	18 (62)	11 (38)	0.045 (0.01, 0.23)	<0.001
	Placebo n = 29	n (%)	2 (7)	27 (93)		

C.I. = Confidence Interval
 * Non-zero includes 1 (mild), 2 (moderate), and 3 (severe)
 ** p-value based on a 2x2 Chi-Square test
 Note: A single missing observation for a patient at Week 20 was assigned a Score = 3.

An illustration of the typical level of observed GL-3 clearance from the kidney interstitial capillary endothelial cells after treatment with Fabrazyme is presented in Figure 4-2. Persistence and confirmation of this effect was also seen in multiple cell types in the Extension study and in patients who crossed over from Placebo to Fabrazyme (see Section 4.2.1.1 and 4.2.1.2).

Figure 4-2 Renal Interstitial Capillary Endothelial Cells are Cleared of GL-3 after Enzyme Replacement Therapy (Score =3 Cleared to Score = 0)

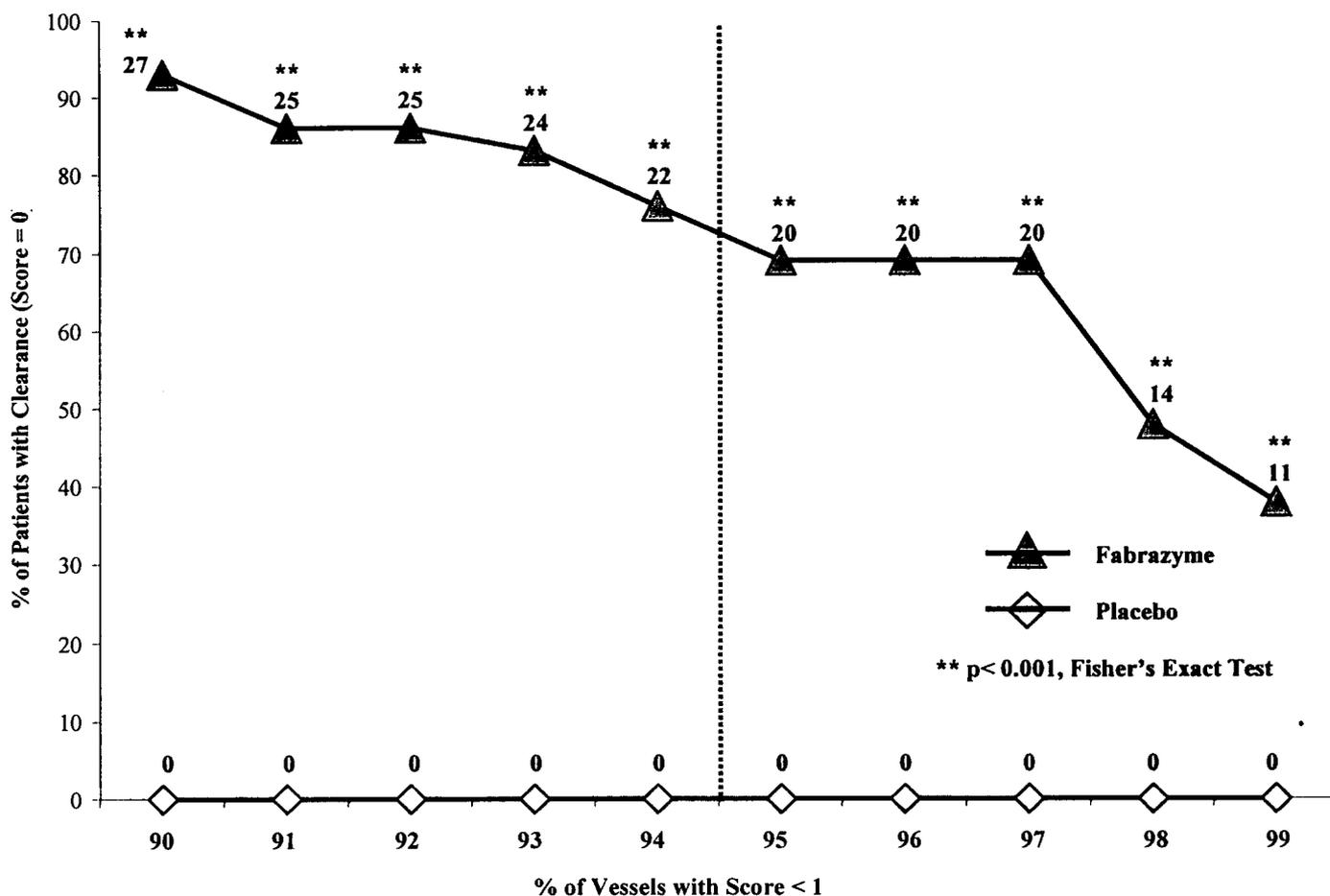


Histologic section of kidney using light microscopy (Richardson's stain, 1000x magnification). Left panel is from a patient biopsy at Baseline. Arrows point to interstitial capillary endothelial cells laden with abnormal lysosomal accumulation of GL-3. Right panel is a biopsy from the same patient following 20 weeks of treatment with Fabrazyme. Arrows point to interstitial capillary endothelial cells that now appear normal.

4.1.1.1 Primary Endpoint Sensitivity Analysis

As noted in Figure 3-4 (scoring system), for a zero-score at least 95% of interstitial capillary endothelial cells were required to have a score < 1. As noted in Section 4.1.1, this endpoint was achieved with a high degree of statistical significance. In order to assess the robustness of this result, a sensitivity analysis was undertaken. The analysis showed that this result was still statistically significant even if at least 99% of interstitial capillary endothelial cells were required to have a score less than 1 ($p < 0.001$). (Figure 4-3)

Figure 4-3 Sensitivity Analysis of Kidney LM Results at Week 20

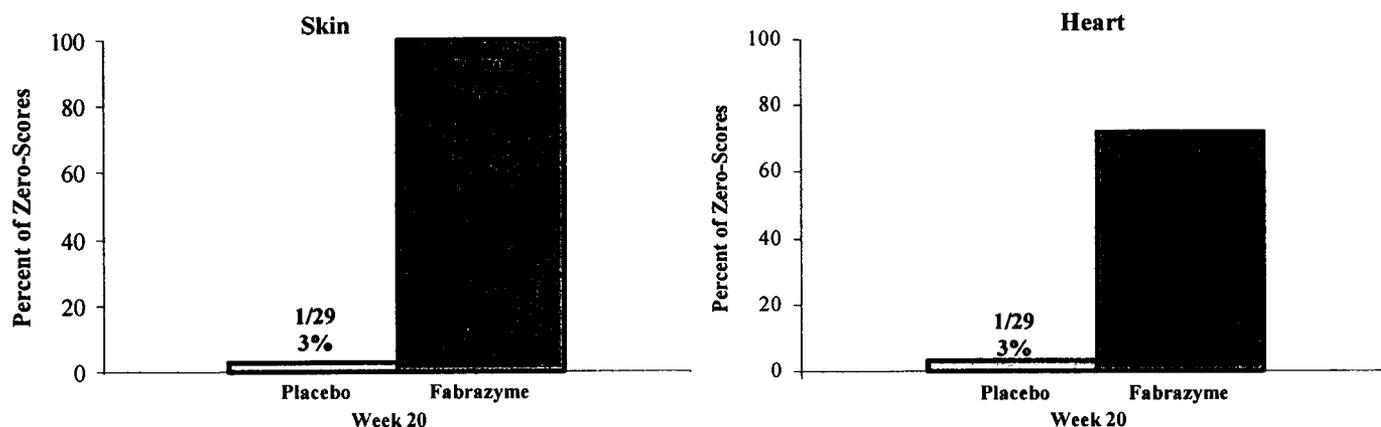


4.1.2 Secondary Endpoints

4.1.2.1 Capillary Endothelium of the Kidney, Skin, and Heart

The percentages of patients achieving zero-score at Week 20 for the interstitial capillary endothelial cells of the skin and heart are displayed for both treatment groups in Figure 4-4. The percent of zero-scores in the interstitial capillary endothelial cells of the skin and heart tissues were similar to those observed for the primary endpoint, kidney tissue. (Figure 4-1)

Figure 4-4 Percent Zero-Scores in the Interstitial Capillary Endothelial Cells of the Skin and Heart (As Treated Population)



A composite scoring method was used to display the mean change in GL-3 clearance from capillary endothelium of the kidney, heart, and skin from Baseline to Week 20 using light microscopy. Each organ was scored by three groups of independent renal, cardiac, and skin pathologists. The majority score was calculated per organ and then summed across all organs. Each composite score ranged from 0 - 9. The mean change in this composite score was calculated and compared between Baseline and Week 20. (Table 4-2 and Figure 4-5)

The marked clearance to normal or near-normal levels of the GL-3 accumulation in the interstitial capillary endothelial cells of each organ is highly statistically significant when analyzed individually and when analyzed according to the prospectively defined composite endpoint ($p < 0.001$).

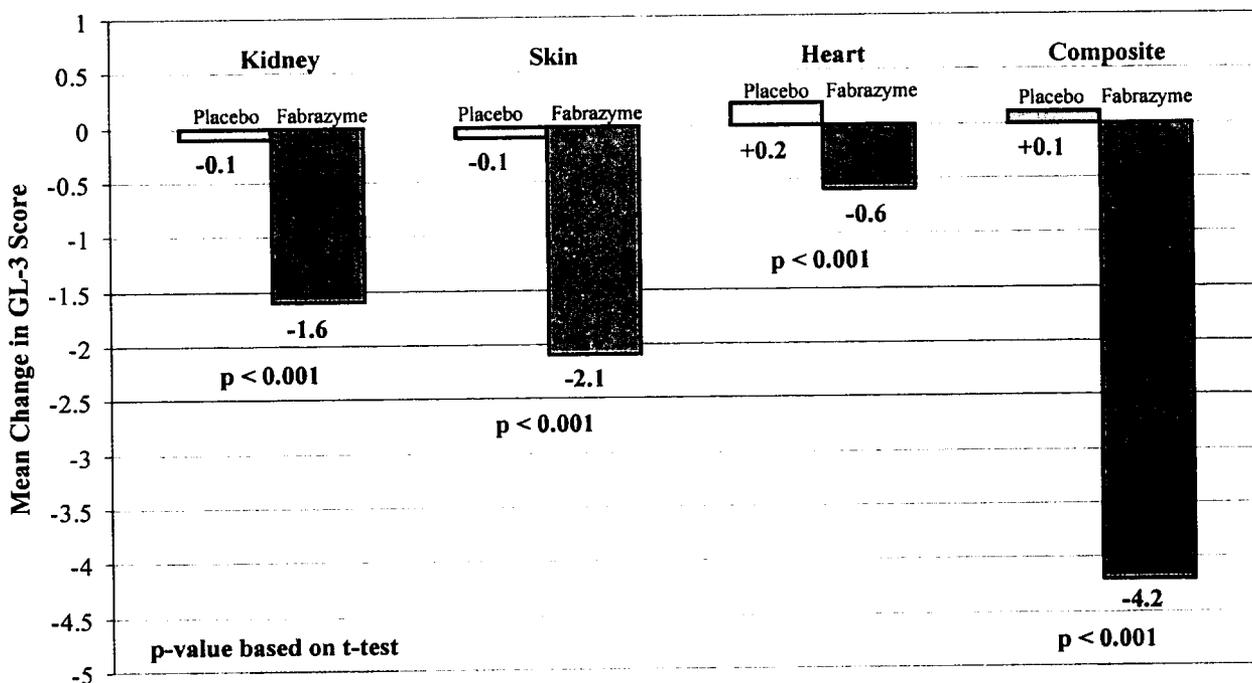
Additional analyses on multiple additional cell-types were subsequently conducted to verify that clearance of GL-3 accumulation was not an isolated finding limited to interstitial capillary endothelial cells. These analyses are presented in Section 4.2.1.2.

Table 4-2 Summary of the Change in the Assessments of GL-3 in the Kidney, Skin, and Heart Using LM: As Treated Population

Organ	Treatment Group	Statistic	Baseline	Week 20
Kidney	Placebo	n	29	29
		Mean ± SD	2.2 ± 0.68	2.1 ± 0.79
	Fabrazyme	n	29	29
		Mean ± SD	1.9 ± 0.80	0.4 ± 0.68
Skin	Placebo	n	29	29
		Mean ± SD	2.3 ± 0.80	2.2 ± 0.71
	Fabrazyme	n	29	29
		Mean ± SD	2.1 ± 0.74	0.0 ± 0.00
Heart	Placebo	n	29	29
		Mean ± SD	0.9 ± 0.53	1.2 ± 0.60
	Fabrazyme	n	29	29
		Mean ± SD	0.9 ± 0.44	0.3 ± 0.54
Composite	Placebo	n	29	29
		Mean ± SD	5.4 ± 1.35	5.5 ± 1.62
	Fabrazyme	n	29	29
		Mean ± SD	4.9 ± 1.53	0.7 ± 0.85

Note: Composite = the sum of the majority scores for kidney, skin, and heart.

Figure 4-5 Summary of the Mean Change from Baseline to Week 20 in the Assessments of GL-3 in the Kidney, Skin, and Heart Using LM: As Treated Population



4.1.2.2 Urinary and Kidney Tissue GL-3 Levels

For GL-3 levels in urine and kidney tissue, the percent change was compared between treatment groups using the rank sum score for each patient. The change score was calculated, ranked, and then summed together as defined prospectively.

Median urine GL-3 decreased by 23.3% in the Fabrazyme treatment group and increased by 42.8% in the placebo group. The median kidney tissue GL-3 decreased by 34% in the Fabrazyme group and decreased by 6.2% in the placebo group. As shown in Table 4-3, the distribution of rank sum scores between the two treatment groups was statistically significant ($p = 0.003$).

Table 4-3 Rank Sum Score of Percent Change in GL-3 Accumulation from Baseline to Week 20 in Urine and Kidney Tissue Measured by ELISA (As Treated Population)

Parameter	Statistic	Fabrazyme	Placebo	P-Value*
Urine GL-3 % Change	n	21	21	0.005
	Median	-23.3	42.8	
Kidney Tissue GL-3 % Change	n	27	28	0.256
	Median	-34.1	-6.2	
Rank Sum Score	n	20	21	0.003
	Median	32.5	48.0	

Note: Urine GL-3 was measured in nmoles/filter and kidney tissue GL-3 in ng/mg
* p-value = p-value for Wilcoxon's Rank Sum Test
Note: Rank Sum Score = Sum of ranked change scores for kidney and urine.
Note: Urinary GL-3 determinations at two of the eight sites were compromised due to logistical issue and are, therefore, not included in the analysis. Additionally, data from Patient 307 are excluded from the analysis because of a missing kidney biopsy from Week 20.

In addition to the analysis of GL-3 levels in kidney tissue and urine, the relationship between the three biochemical parameters that were measured (urinary, kidney, and plasma GL-3 levels) and kidney LM scores was examined. (Section 4.1.3.2)

4.1.2.3 Pain Measured by the Short Form McGill Pain Questionnaire

The secondary endpoint of pain was measured by the Short Form McGill Pain Questionnaire. In addition, tertiary endpoints of pain measurements used the Present Pain Index (PPI) and Visual Analog Scale (VAS). The Short Form McGill Pain Questionnaire, PPI, and VAS permit scoring of pain based on the scale summarized in Table 4-4.

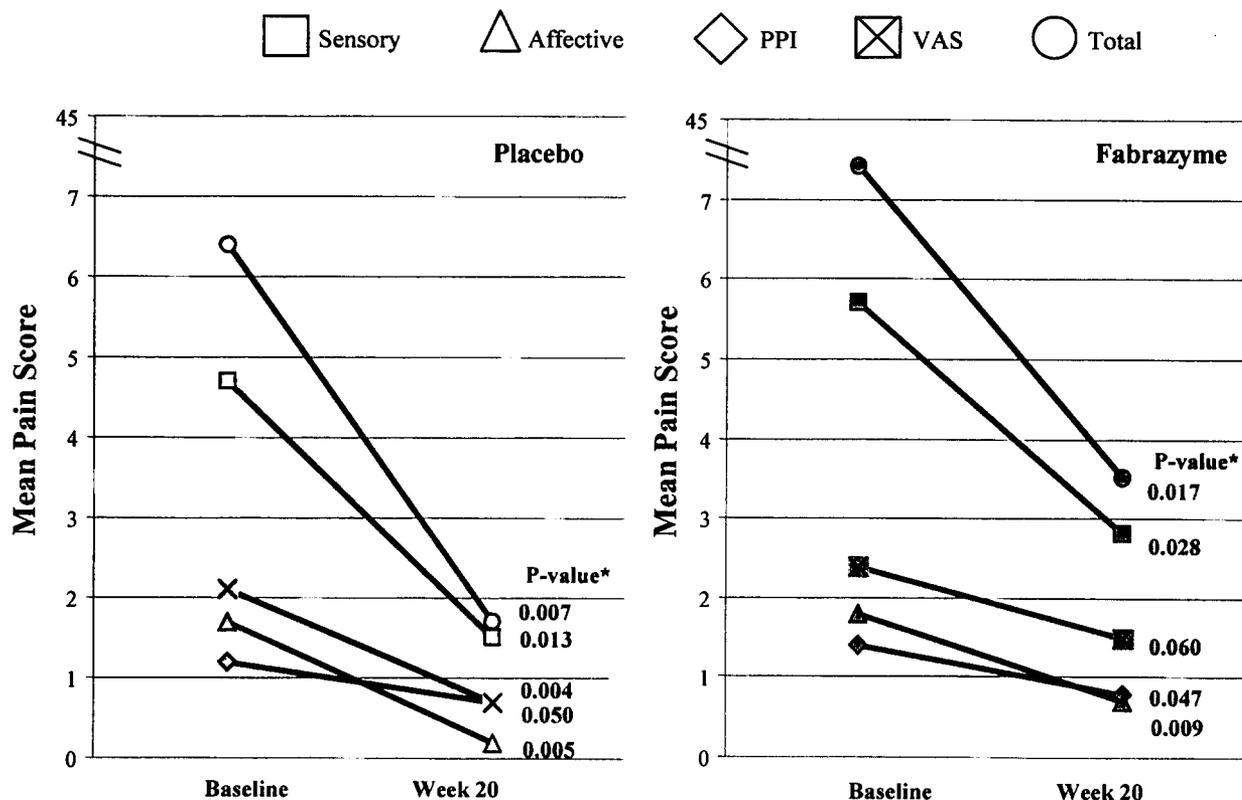
Table 4-4 Pain Scoring Scales

Pain Parameter	Scale (Range)
Short Form McGill Pain Questionnaire (Secondary Endpoint)	
Sensory	0 – 33
Affective	0 – 12
Total	0 – 45
Present Pain Intensity (PPI) (Tertiary Endpoint)	0 – 5
Visual Analog Scale (Tertiary Endpoint)	0 – 10
Lower number = less pain Higher number = more pain	

Values observed at Baseline in each treatment group were relatively low (less pain) and were not different from each other for each of the parameters measured using the Short Form McGill Pain Questionnaire. The Phase 3 Placebo-Controlled Study, while assessing pain as a secondary endpoint, was not designed as a pain study with respect to sample size. In addition, patients were not selected on the basis of severe pain, no effort was made to standardize pain medications, and patients were not asked to wean or abstain from their pain medications during the course of the study.

There was no significant difference between treatment groups in pain parameters, possibly due to a strong placebo effect although improvement in multiple pain parameters were noted within treatment groups in the Phase 3 Placebo-Controlled Study. This improvement has been maintained for up to 24 months of Fabrazyme therapy. (Figure 4-6) It is not surprising to observe a confounding placebo effect in a relatively small study. Most pain studies are much larger than the Phase 3 Placebo-Controlled Study. Conducting such an appropriately sized study to assess parameters as subjective as pain is problematic in a disease with prevalence as low as that of Fabry disease and with the significant heterogeneity that exists in the clinical presentation and severity of pain.

Figure 4-6 Mean Pain Rating Values of the Short Form McGill Pain Questionnaire



* p-value: One sample t-test

4.1.3 Tertiary and Other Endpoints

4.1.3.1 Plasma GL-3

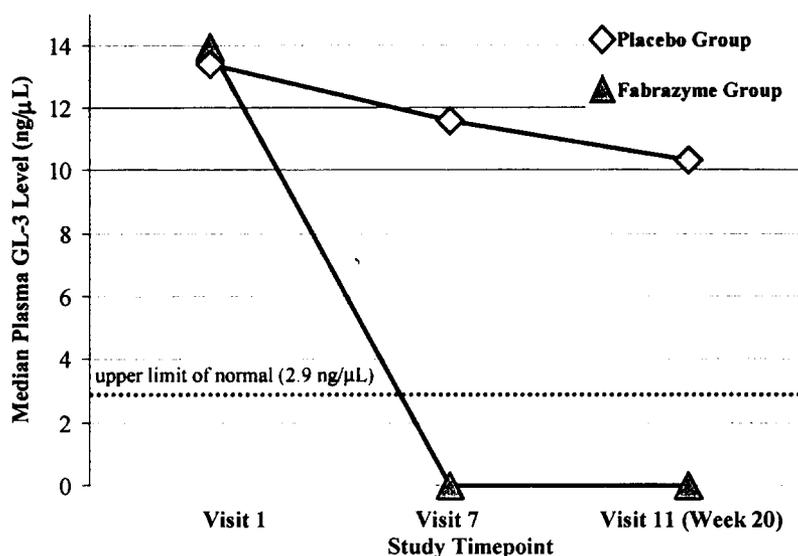
Plasma GL-3 levels were measured (by ELISA) as an indicator of αGAL activity and an indirect marker of the level of Fabrazyme reaching the different cells with accumulated GL-3.

It is important to understand that just like the naturally occurring lysosomal hydrolase, Fabrazyme has a pH optimum of approximately 4.5, almost three orders of magnitude more acidic than the pH of plasma, which is approximately 7.4. Fabrazyme is essentially inactive in plasma. Therefore, the clearance of plasma GL-3 resulting from treatment with Fabrazyme appears to represent decreased egress from body tissues as a result of effective intracellular enzyme replacement therapy. While clearance of plasma GL-3 may not be sufficient, it seems reasonable to postulate that clearance may be necessary to significantly impact the underlying Fabry disease pathology and that the level of plasma GL-3 may provide a measure of the total tissue load of GL-3.

For Fabrazyme-treated patients, a highly statistically significant difference was observed in percent change from Baseline for median plasma GL-3 ($p < 0.001$). In the Phase 3 Placebo-Controlled study, the Baseline median plasma GL-3 level for the Fabrazyme treatment group was 13.9 ng/ μ L and for the placebo group was 13.4 ng/ μ L. A statistically significant difference was observed for percent change from Baseline for median plasma GL-3. At Week 20 the median percent change from Baseline value in the Fabrazyme treatment group decreased by 100% while the median value in the placebo treatment group decreased by 16.5%.

Figure 4-7 illustrates the decrease in median plasma GL-3 levels of patients after treatment with Fabrazyme.

Figure 4-7 Median Plasma GL-3 Levels in the Phase 3 Placebo-Controlled Study

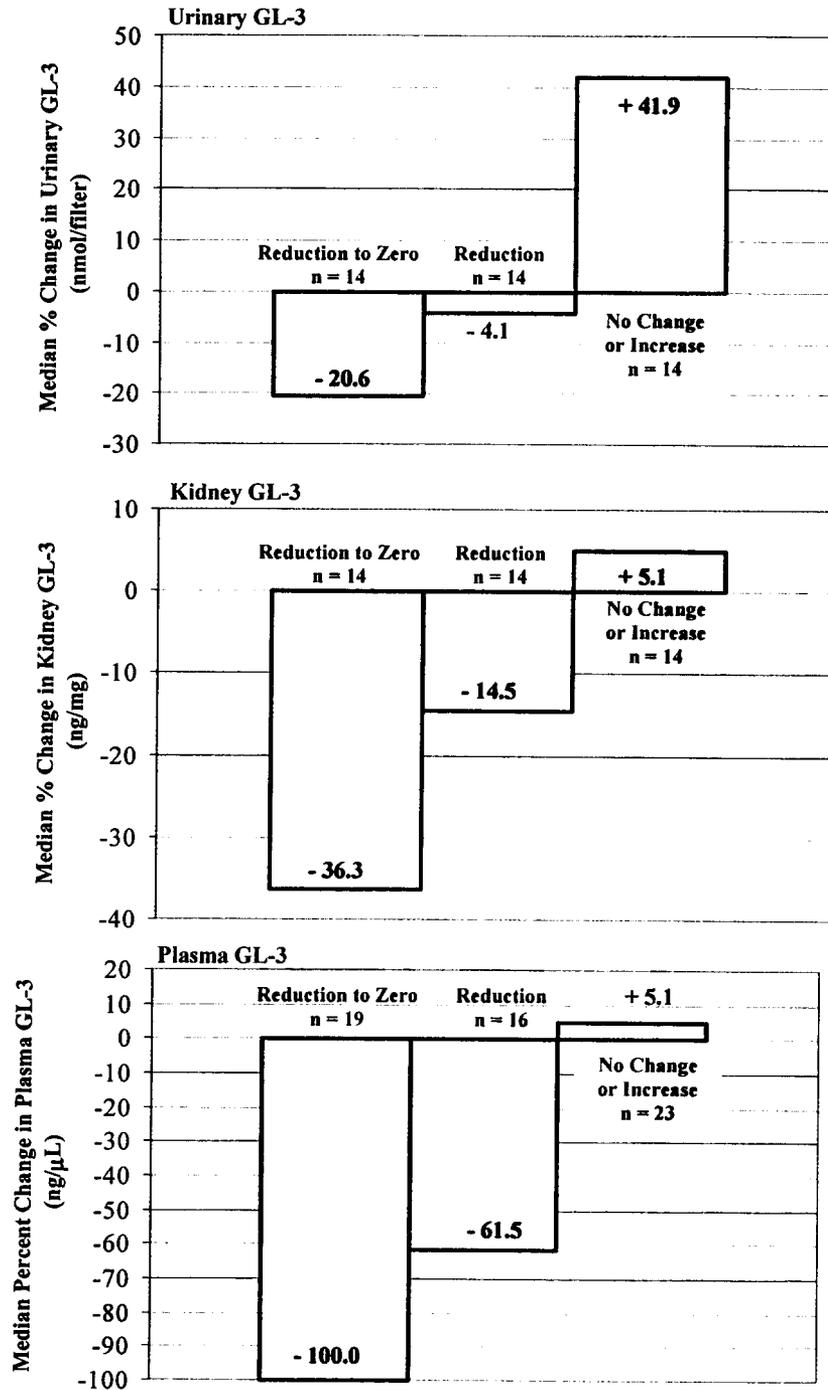


4.1.3.2 Relationship Between Biochemical and Histologic Clearance of GL-3

An additional analysis was conducted to examine the relationship between the histology scores and the GL-3 ELISA measurements of kidney tissue, urine, and plasma. (Figure 4-8)

Patients were categorized according to their histologic response to treatment with Fabrazyme (i.e., Reduction to Zero, Reduction, and No Change or Increase). This analysis showed that a relationship can be seen between reduction in LM kidney scores and the biochemical measurements that were performed. The greater the reduction in kidney LM score, the greater the observed median percent decrease in GL-3 in urine, kidney tissue, and plasma. The strongest relationship was observed in plasma. This analysis further supports the histologic findings.

Figure 4-8 Relationship Between Kidney LM Scores and Biochemical Measures (GL-3) (Both Treatment Groups Combined) (Note: n = total number of patients with both a LM score and an ELISA GL-3 assessment value for the corresponding biochemical measurement)



4.1.3.3 Renal Function

Average renal function as assessed by serum creatinine was normal in both groups at Baseline and did not change significantly in either treatment group during the study. Serial mean serum creatinine measurements are shown in Table 4-5.

Table 4-5 Mean Serum Creatinine (mg/dL)

Treatment Group	Statistic	Baseline	Week 20
Placebo (n = 29)	mean ± sd	0.8 ± 0.21	0.8 ± 0.26
Fabrazyme (n = 29)	mean ± sd	0.8 ± 0.20	0.9 ± 0.24

Glomerular filtration rate (GFR) was determined by a measure of inulin clearance. However, the commercial supplier discontinued the manufacture of inulin during the study. Therefore, 57/58 patients had inulin clearance assessments at Baseline, only 36 patients had assessments performed at Week 20. Table 4-6 summarizes GFR results for those patients who had inulin clearance assessments performed.

Table 4-6 Glomerular Filtration Rate as Determined by Inulin Clearance (mL/min/1.73m²)

Visit	Statistic	Treatment Group	
		Placebo	Fabrazyme
Baseline	n	28	29
	mean ± sd	97.4 ± 34.74	82.3 ± 22.23
Week 20	n	19	17
	mean ± sd	100.6 ± 34.77	93.9 ± 36.15

4.2 Phase 3 Extension Study

Since Fabrazyme is intended for long-term enzyme replacement therapy for patients with Fabry disease, it is essential that long-term follow-up data are collected on patients who receive Fabrazyme. Therefore, at the completion of the Phase 3 Double-Blind Placebo-Controlled Study, a Phase 3 Open-Label Extension Study was initiated that investigated many of the same endpoints of the Placebo-Controlled Study. All 58 patients from the original study elected to enroll in the long-term extension study, which was designed to treat all enrolled patients with Fabrazyme on an open-label basis and to monitor the patients for an additional 4.5 years after completing the Placebo-Controlled Study.

In order to differentiate between the beginning of the two studies:

- “Baseline” was used to identify the beginning timepoint of the Phase 3 Placebo-Controlled Study,
- “Entry” was used to identify the beginning timepoint of the Phase 3 Extension Study.

Efficacy (and Safety) measurements are conducted every 6 months and are discussed relative to “Baseline” and to “Entry.”

Patients who received placebo in the Phase 3 Placebo-Controlled Study switched to treatment with Fabrazyme in the Phase 3 Extension Study. These patients are designated as “Placebo/Fabrazyme” (“PL/FZ”) patients. Similarly, patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study are designated as “Fabrazyme/Fabrazyme” (“FZ/FZ”) patients.

The difference between Baseline and Entry becomes most important when considering the time on treatment for the patients in the Phase 3 Extension Study. In the Phase 3 Extension Study Placebo/Fabrazyme patients have received approximately 20 fewer weeks of treatment with Fabrazyme than the Fabrazyme/Fabrazyme patients.

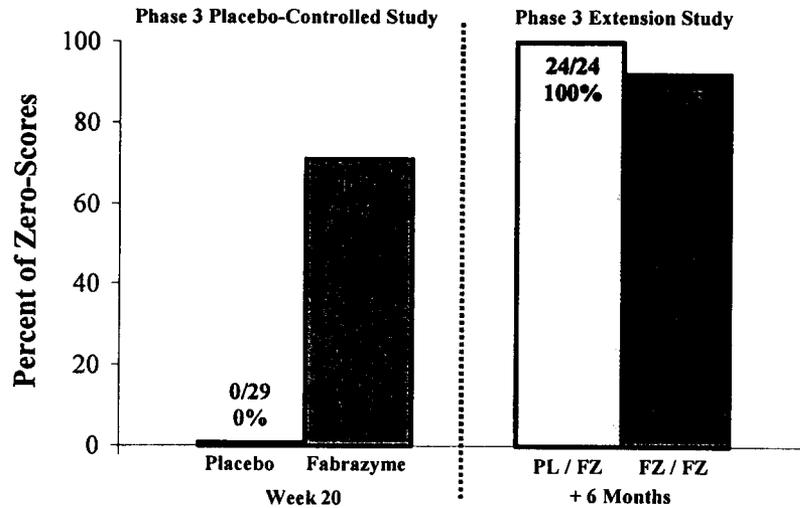
4.2.1 Study Results

4.2.1.1 Histologic Assessment of Interstitial capillary endothelial Cells of Kidney, Skin, and Heart

After 6 months of open-label treatment with Fabrazyme in the Phase 3 Extension Study, the kidney, heart, and skin interstitial capillary endothelial cell response of patients who originally received placebo in the Phase 3 Placebo-Controlled Study was similar to the response of patients who received treatment with Fabrazyme in that study. (Figure 4-9, Figure 4-10, and Figure 4-11, respectively)

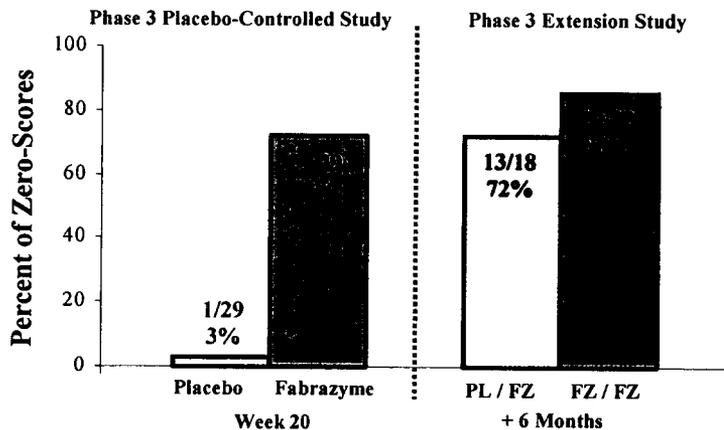
For skin tissue biopsies, efficacy assessments were conducted every 6 months through the 18-month timepoint of the Phase 3 Extension Study and then yearly thereafter. For kidney and heart tissue, the challenges of repeat biopsies limited additional assessments to the 6-month timepoint only.

Figure 4-9 Kidney Histology: Response of Interstitial capillary endothelial Cells in the Phase 3 Placebo-Controlled Study and Phase 3 Extension Study



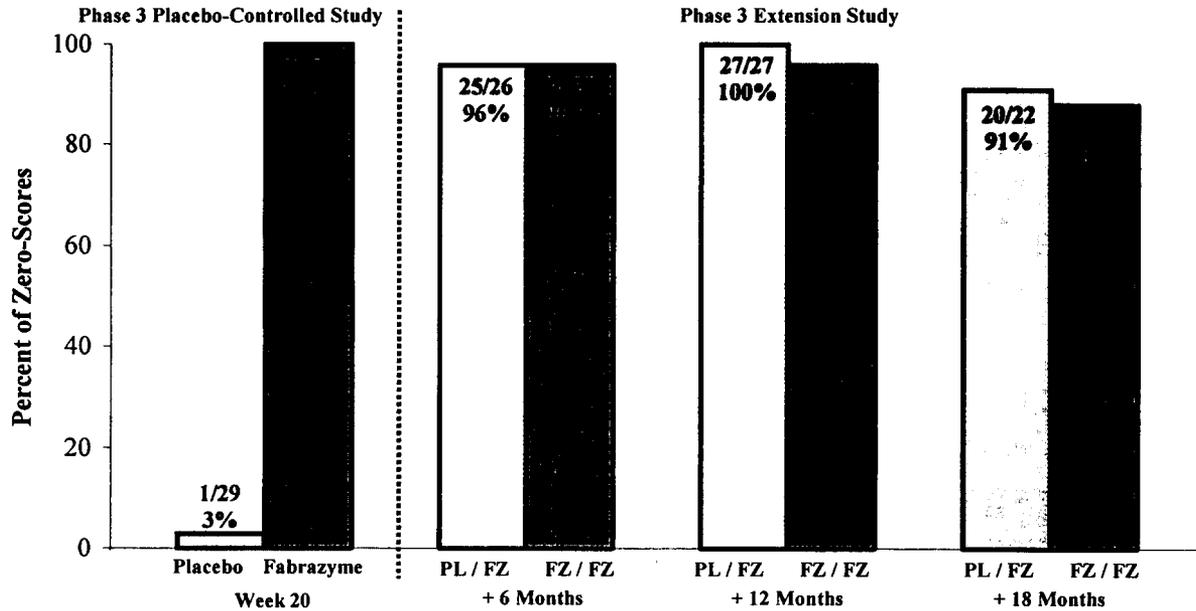
PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

Figure 4-10 Heart Histology: Response of Interstitial capillary endothelial Cells in the Phase 3 Placebo-Controlled Study and through 6 Months of Open-Label Treatment with Fabrazyme in the Phase 3 Open-Label Extension Study



PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

Figure 4-11 Skin Histology: Response of Interstitial capillary endothelial Cells in the Phase 3 Placebo-Controlled Study and through 18 Months of Open-Label Treatment with Fabrazyme in the Phase 3 Extension Study

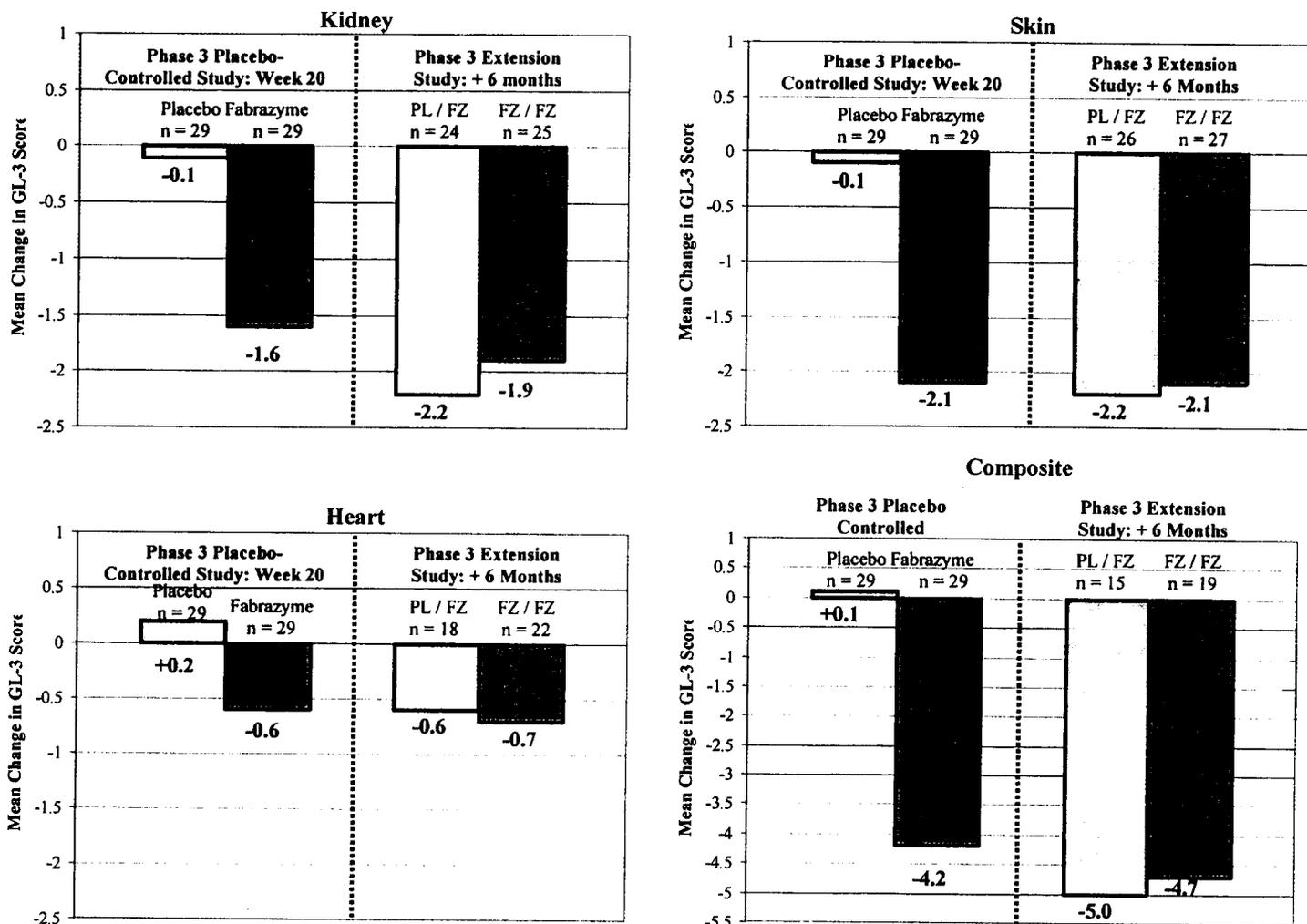


PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

The mean change in the LM scores for kidney, skin, and heart and the composite LM tissue score is provided by treatment group from Baseline to Week 20 of the Phase 3 Placebo-Controlled Study and to 6-months of the Phase 3 Extension Study Figure 4-12.

When patients who received placebo in the Phase 3 Placebo-Controlled Study were switched to treatment with Fabrazyme in the Phase 3 Extension Study, these patients showed large mean decreases in tissue scores. These large mean decreases were similar in magnitude to the decreases observed in patients who had received treatment with Fabrazyme in the Phase 3 Placebo-Controlled Study, confirming the impact of Fabrazyme on interstitial capillary endothelial cell GL-3 clearance.

Figure 4-12 Summary of the Mean Change from Baseline in the Assessments of GL-3 in the Kidney, Skin, and Heart Using LM: As Treated Population



PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

After 18 months into the Phase 3 Extension Study, 41/46 (89%) patients had a score of zero in skin interstitial capillary endothelial cells. This result was statistically significant. However, five patients had non-zero scores for skin capillary endothelium at the 18-month timepoint. Four of the five patients that had non-zero skin scores underwent subsequent biopsies and now have zero scores. (Table 4-7) Based on the results of the subsequent skin biopsies there appears to be no evidence for reoccurrence of substrate deposition. Thus, the non-zero scores at 18 months most likely represent sampling differences and do not indicate a true reaccumulation of substrate. We agree with FDA that a “change in score by 1 point does not reliably indicate a true change” (FDA Advisory Committee Briefing Document, August 26, 2002, Page 26).

Table 4-7 Summary of Skin Biopsy Scores at Each Study Timepoint (As Treated Population)

Patient ID	Treatment Group	Phase 3 Placebo-Controlled Study		Phase 3 Extension Study				Remarks
		Baseline Score	Week 20 Score	6 Month Score	1 Year Score	18 Month Score	30 Month Score	
102	PL/FZ	2	2	0	0	1	0	serum creatinine stable
107	FZ/FZ	2	0	0	0	1	0	serum creatinine stable
201	PL/FZ	3	2	0	0	1	0	serum creatinine stable
705	FZ/FZ	2	0	0	1	2	0**	serum creatinine stable; serum IgE positive (successfully received subsequent infusions)
806	FZ/FZ	2	0	0	0	1	N/A†	serum creatinine stable; 10 weeks elapsed between last infusion and 18-Month biopsy; skin-test positive;(successfully received subsequent infusions)

PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study
 **Biopsy done at 2 years post-entry into extension (at time of withdrawal from the study)
 †Repeat biopsy not yet available

4.2.1.2 Histologic Assessment of Additional Cell-Types of the Kidney and Skin

In response to questions from FDA to verify that GL-3 clearance in capillary endothelial cells was not an isolated finding, additional kidney and skin cell-types were identified for analysis of response to long-term treatment with Fabrazyme. These additional cell-types were retrospectively

evaluated in the pathology slides obtained at Baseline and Week 20 of the Phase 3 Placebo-Controlled Study and 6 Months into the Phase 3 Extension Study.

The additional kidney cell-types for analysis included:

- Glomerular endothelial cells
- Non-capillary (arteriolar) smooth muscle cells
- Non-capillary (arteriolar) endothelial cells
- Podocytes
- Distal convoluted tubules/collecting ducts
- Mesangial cells
- Interstitial Cells

The additional skin cell-types for analysis included:

- Deep vessel endothelial cells
- Deep vessel smooth muscle cells
- Cells of the perineurium

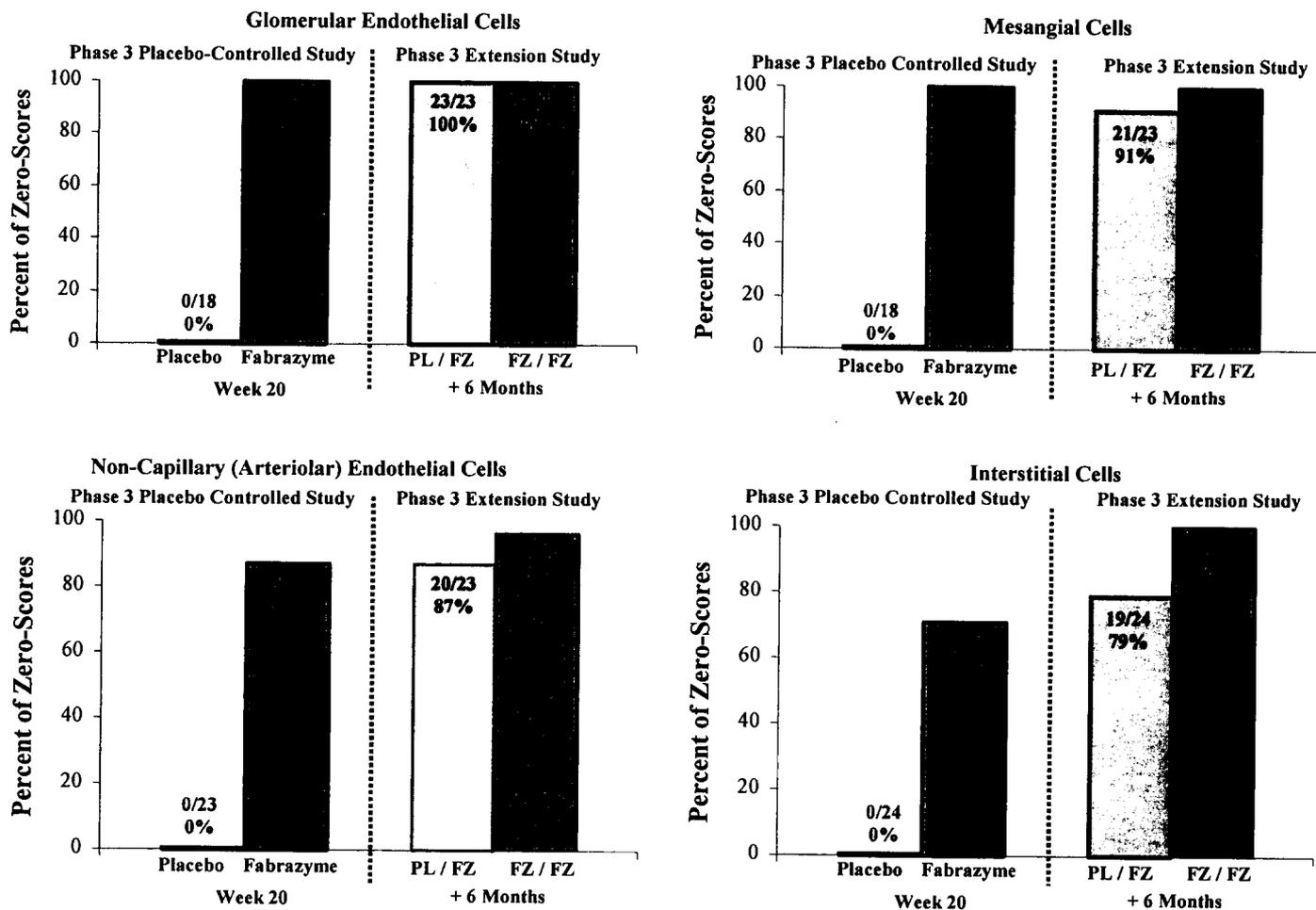
At the end of the Phase 3 Placebo-Controlled Study (Week 20), for several additional kidney cell-types, there was a highly statistically significant difference ($p < 0.001$) between the Fabrazyme and placebo treatment groups in the number of patients achieving a zero-score. This occurred in the Fabrazyme group for the following kidney cell-types:

- Glomerular endothelial cells
- Mesangial cells
- Non-capillary (arteriolar) endothelial cells
- Interstitial cells

Nearly all of the patients with available data who had originally received placebo in the Placebo-Controlled study showed clearance to near-normal levels of GL-3 accumulation in these additional kidney cell-types after 6 months of open-label treatment with Fabrazyme.

The results for these kidney cell-types are presented graphically in Figure 4-13.

Figure 4-13 Effect of Fabrazyme in the Phase 3 Extension Study for Kidney Cell-Types that Showed Statistically Significant Differences ($p < 0.001$) in Zero-Scores Between Placebo and Fabrazyme Treatment Groups in the Phase 3 Placebo-Controlled Study



PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

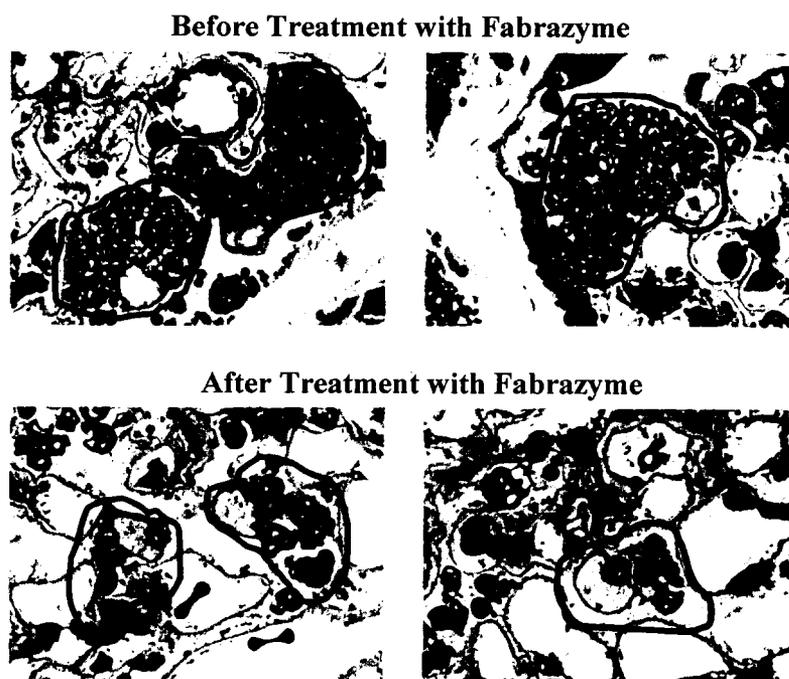
For other kidney cell-types, Fabrazyme reduced GL-3 levels, albeit not to zero. The kidney cell-types included podocytes (Figure 4-14), distal convoluted tubules/collecting ducts, and non-capillary interstitial smooth muscle cells. (Table 4-8)

Table 4-8 Kidney Histology: Response of Podocytes, Distal Convoluted Tubules/Collecting Ducts, and Non-Capillary Smooth Muscle Cells from Baseline of the Phase 3 Placebo-Controlled Study through 6 Months of Open-Label Treatment with Fabrazyme in the Phase 3 Extension Study

Renal Cell-Type	Placebo/Fabrazyme		Fabrazyme/Fabrazyme	
	Patients with GL-3 Scores Reduced		Patients with GL-3 Scores Reduced	
	Baseline to Week 20	Baseline to 6 Months	Baseline to Week 20	Baseline to 6 Months
Podocytes	0/16 (0%)	5/22 (23%)	1/19 (5%)	3/17 (18%)
Distal Convoluted Tubules/Collecting Ducts	1/24 (4%)	18/24 (75%)	6/24 (25%)	12/24 (50%)
Non-Capillary Smooth Muscle Cells*	2/22 (9%)	19/22 (86%)	18/21 (86%)	17/21 (81%)

* GL-3 scores reduced by 1 or 2 points from Baseline score but did not reach a score of 0.

Figure 4-14 Sample Histology Slide of GL-3 Reduction in Podocytes for One Patient after 12 Months of Treatment with Fabrazyme (Podocytes Circled)

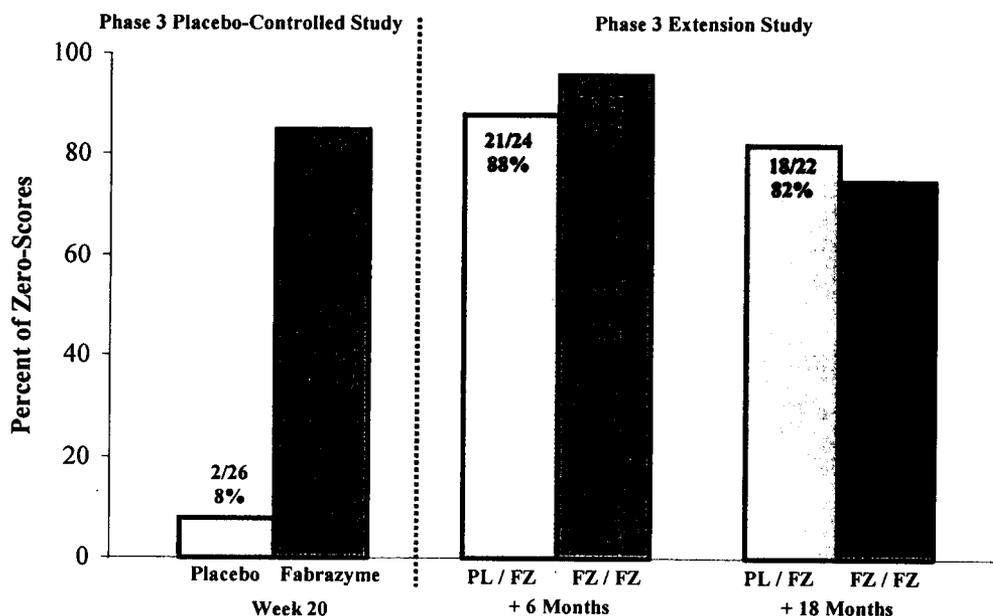


Histologic section of kidney using light microscopy (Richardson's stain, 1000x magnification)

At the end of the Phase 3 Placebo-Controlled Study, for the skin deep-vessel capillary endothelial cells, there was a statistically significant difference ($p < 0.001$) between the Fabrazyme and placebo treatment groups. When patients who received placebo in the Phase 3 Placebo-

Controlled Study were switched to treatment with Fabrazyme in the Phase 3 Extension Study, these patients showed large mean decreases in skin tissue scores for deep-vessel capillary endothelial cells. The results for these skin cell-types are presented graphically in Figure 4-15.

Figure 4-15 Skin Histology: Effect of Fabrazyme in the Phase 3 Extension Study for Deep-Vessel Capillary Endothelial Cells Showing Statistically Significant Differences ($p < 0.001$) in Zero-Scores Between Placebo and Fabrazyme Treatment Groups in the Phase 3 Placebo-Controlled Study



PL/FZ (Placebo/Fabrazyme) – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

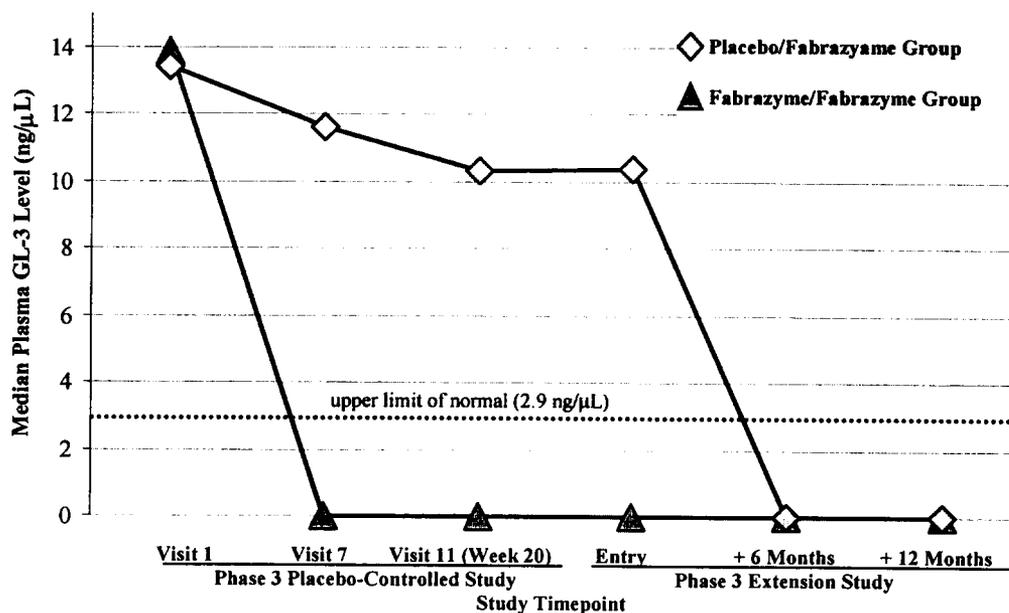
FZ/FZ (Fabrazyme/Fabrazyme) – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

The deep-vessel smooth muscle cells and cells of the perineurium appear to respond more slowly than the capillary endothelial cells of the skin. However, conclusions drawn on the observed results should be made cautiously since these are superficial skin biopsies that do not always contain sufficient deep cell structures for analysis.

4.2.1.3 Plasma GL-3

The change in median plasma GL-3 levels obtained for patients who originally received placebo in the Phase 3 Placebo-Controlled Study and who then switched to Fabrazyme treatment in the Phase 3 Extension Study were similar to those obtained for patients who originally received Fabrazyme in the Phase 3 Placebo-Controlled Study. (Figure 4-16)

Figure 4-16 Median Plasma GL-3 Levels in the Phase 3 Placebo-Controlled Study and Phase 3 Extension Study



Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

At the time of entry to the open-label study, the median of plasma GL-3 was below the level of detection (< 1.2 ng/μL) for patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and was 10.4 ng/μL for patients who had received placebo in the Phase 3 Placebo-Controlled Study. Patients treated with Fabrazyme during both the Placebo-Controlled and Extension studies maintained their lowered plasma GL-3 levels for an additional 1 year. Patients treated with placebo prior to open-label treatment with Fabrazyme showed a median decrease of 100.0% during the 1 year of treatment with Fabrazyme.

4.2.1.4 Renal Function

Glomerular filtration rate (GFR) was determined by a measure of inulin clearance. However, the commercial supplier discontinued the manufacture of inulin during the study. Due to the unavailability of inulin at some timepoints, not all patients were assessed for GFR as determined by inulin clearance at timepoints where there was a shortage of inulin. Therefore Table 4-9 summarizes GFR results at Baseline and at the 12-month timepoint. This is the timepoint at which the maximum number of patients had the inulin assessment performed as compared to

those at Baseline. As shown, GFR remained stable relative to Baseline in both treatment groups at the 12-month timepoint of the Phase 3 Extension Study.

Table 4-9 Glomerular Filtration Rate as Determined by Inulin Clearance (mL/min/1.73m²)

Study	Visit	Statistic	Treatment Group	
			Placebo	Fabrazyme
Phase 3 Placebo-Controlled Study	Baseline	n	28	29
		mean ± sd	97.4± 34.74	82.3 ± 22.23
Phase 3 Extension Study	12 Months	n	22	28
		mean ± sd	100.7± 32.53	79.8± 25.81

During 24 months of follow-up, the mean renal function as assessed by serum creatinine has remained normal. (Table 4-10)

Table 4-10 Mean (±SD) Serum Creatinine Levels (mg/dL)

Treatment Group	Phase 3 Placebo-Controlled Study		Phase 3 Extension Study			
	Baseline	Week 20	6 Months	12 Months	18-Months	24-Months
Placebo/Fabrazyme	0.8 ± 0.2 (n = 29)	0.8 ± 0.3 (n = 29)	0.8 ± 0.3 (n = 26)	0.8 ± 0.3 (n = 28)	0.9 ± 0.3 (n = 27)	0.8±0.4 (n = 25)
Fabrazyme/Fabrazyme	0.8± 0.2 (n = 29)	0.9 ± 0.2 (n = 29)	0.9 ± 0.2 (n = 28)	0.9 ± 0.3 (n = 28)	0.9 ± 0.3 (n = 28)	0.9±0.4 (n = 25)

Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

Ten of the 58 patients in the Phase 3 Study had low estimated GFR at start-of-treatment (defined as < 90 ml/min/1.73 m²). GFR was estimated using an equation from the Modification of Diet in Renal Disease Study. (Levey, 2000, JASN) Eight of 10 (80%) patients in this subset with low GFR, but without pronounced glomerulosclerosis and proteinuria at Baseline, exhibited an improvement or stabilization of GFR at the 24-month timepoint in the Phase 3 extension study. Since Fabry disease is a progressive disease and most patients develop end-stage renal disease in the fourth decade of life, the fact that most patients with low estimated GFR values have shown stabilization is encouraging. The two patients who had worsening of estimated GFR (119 and 804) are discussed in greater detail below.

During active treatment with Fabrazyme, three patients have shown a > 33% increase in serum creatinine from start of treatment with their last value being above normal (> 1.4 mg/dL). (Table 4-11)

Table 4-11 Patients with a > 33% Increase in Serum Creatinine Levels (mg/dL)

Patient	Treatment Group	Phase 3 Placebo-Controlled Study		Phase 3 Extension Study			
		Baseline	Week 20	6 Months	12 Months	18-Months	24-Months
707	Fabrazyme/Fabrazyme	0.7	1.3	1.3	1.4	1.6	1.8
804	Fabrazyme/Fabrazyme	1.1	1.2	1.4	1.8	1.9	2.3
119	Placebo/Fabrazyme	1.3	1.6	1.8	2.1	2.2	2.2

Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study

Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study

Given the heterogeneity of Fabry disease, the precise reason for this phenomenon is unclear. However, common factors among these patients include advanced age, severe Baseline renal pathology, and severe proteinuria at baseline. While the mean ages of the placebo and Fabrazyme treatment groups at Baseline was 28.4 years and 32.0 years, respectively, the ages of Patients 119, 707, and 804 were 48, 45, and 42 years, respectively. This is within the age range during which Fabry patients typically develop renal insufficiency (*Branton, 2002, Medicine*). These 3 patients were among a small subset of 6 patients who had baseline kidney biopsies demonstrating prominent glomerular sclerosis (defined as greater than 50% of all observed glomeruli demonstrating either focal segmental or global glomerulosclerosis) and among a small subset of 10 patients with significant proteinuria at Baseline (defined as a urinary protein (mg/dL):urinary creatinine (mg/dL) ratio greater than 1.0).. Additionally, these three patients were the only patients to have a Baseline urinary protein (mg/dL):urinary creatinine (mg/dL) ratio greater than 2. Two of the three patients (119 and 804) also belong to a subset of 10 patients with low (defined as < 90 ml/min/1.73 m²) estimated GFR at Baseline. Age, baseline urinary protein excretion, and baseline renal pathology, may have placed patients 119, 707, 804 and others in the cohort at increased risk for progression of their renal disease, despite treatment with Fabrazyme. It is well known from other models of renal disease that once a critical number of nephrons are damaged, progression to end-stage renal disease is inevitable. These data strongly suggest that treatment before extensive renal damage occurs is critical. Nonetheless, it is also possible that the rate of progression among these 3 patients was slower than would have occurred without Fabrazyme therapy. (Section 4.2.1.5)

4.2.1.5 Progression of Renal Disease in Patients Treated with Fabrazyme Compared to an Untreated Historical Control Population of Patients with Fabry Disease

An analysis was performed to investigate differences in estimated renal events based on serum creatinine for patients treated with Fabrazyme versus a comparable, untreated historical control population of patients with Fabry disease. Data on this comparable historical control population were collected under a prospectively defined protocol. By-patient linear trend regression modeling was used to compare estimated renal event rates from the patients participating in the Phase 3 Placebo Controlled and Extension studies to those from historical control population. Patients from the historical control population meeting both the inclusion/exclusion criteria of the Phase 3 study and who had serum creatinine observations for greater than 6 months were used for the comparison.

This analysis shows encouraging trends in patients receiving Fabrazyme for up to 30 months indicating a slowing in progression of renal functional decline compared to a matched historical database of untreated Fabry patients. Specifically, when a renal event is defined as an increase in serum creatinine of at least 33% over 2 years, the reduction in risk for patients treated with Fabrazyme is estimated at 63%. When a renal event is defined as an increase in serum creatinine of 50% over 3 years, the reduction in risk for patients treated with Fabrazyme is estimated at 50%.

Table 4-12 Two- and Three-Year Event Rate Estimates (33% and 50% Increases, respectively) of Phase 3 Extension Compared to Historical Control

Event Rate Estimate	Estimated Event Rate		P-Value	Odds Ratio and CI	Risk Reduction
	Phase 3 Extension (Fabrazyme)	Historical Control			
2-year/33% Increase	5.3% (3/57)	13% (17/130)	0.124	0.369 (0.104, 1.314)	63%
3-year/50% Increase	7.0% (4/57)	13% (17/130)	0.234	0.502 (0.161, 1.564)	50%

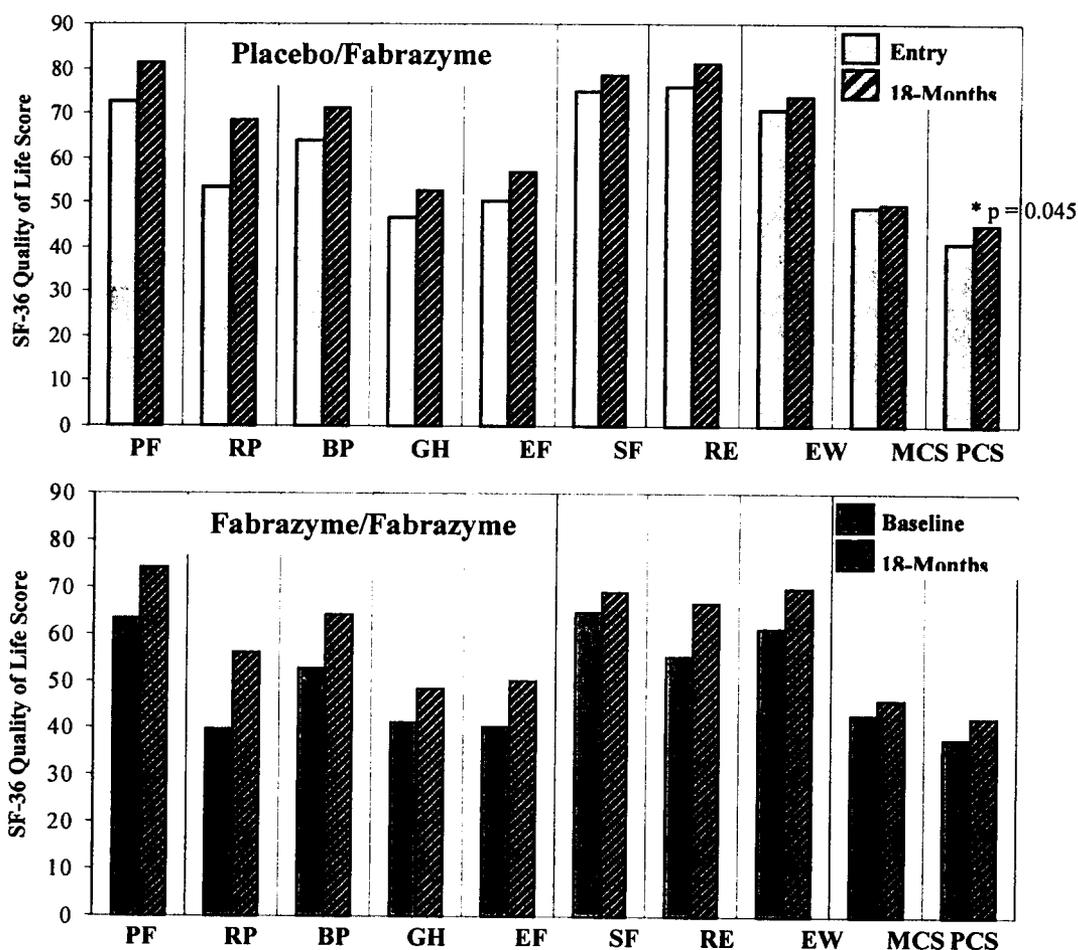
CI = 95% Confidence Interval
Risk Reduction = (1-Odds Ratio)×100

A beneficial trend is observed in that the point estimate for the odds ratio is approximately 0.50, corresponding to a Risk Reduction of 50% for patients treated with Fabrazyme relative to patients in the Historical Control study. It should be noted, however, that although the point estimates are encouraging, these results are not statistically significant. Therefore, it is expected that with a longer follow-up period of Fabrazyme treated patients these initial findings will be confirmed.

4.2.1.6 Quality of Life as Measured by the SF-36 Health Status Survey

At the 18-month timepoint of the Phase 3 Extension Study, an improvement was observed in both treatment groups for most SF-36 parameters. At 18 months, there was a statistically significant change from Entry for the patients who received placebo in the Phase 3 Placebo-Controlled Study in the standardized Physical Component Scale ($p = 0.045$). In addition, the change from Entry for Physical Functioning approached statistical significance for both treatment groups.

Figure 4-17 Quality of Life: Mean SF-36 Health Status Survey Score



Placebo/Fabrazyme – patients who received placebo in the Phase 3 Placebo-Controlled Study and who switched to treatment with Fabrazyme in the Phase 3 Extension Study
Fabrazyme/Fabrazyme – patients who received Fabrazyme in the Phase 3 Placebo-Controlled Study and who continued receiving Fabrazyme in the Phase 3 Extension Study
SF-36 Standardized Physical Component Scale (PCS) categories are: Physical Functioning (PF), Role Physical (RP), Body Pain (BP), and General Health (GH).
SF-36 Standardized Mental Component Scale (MCS) categories are: Energy/Fatigue (EF), Social Functioning (SF), Role Emotional (RE), and Emotional Well-Being (EW).

4.3 Phase 2 Open-Label Study (Japan)

An open-label, bridging study that adhered to the ICH Harmonized Tripartite Guideline (Feb 5, 1998) was completed in Japan on 13 patients. Entry criteria and endpoints were virtually identical to the Phase 3 Placebo-Controlled Study described above. Endpoints included GL-3 clearance from kidney and skin vasculature measured by light microscopy (LM) and ELISA, and Quality of Life improvement. The pathology read for the Japan study was designed as a completely blinded read of all cell types at once. The read of the renal capillary endothelium, the primary endpoint of the Phase 3 Placebo-Controlled Study, was not separated from the read of the additional cell types. Each slide was blinded and evaluated individually.

Patients were 16 – 34 years of age (mean 26.6) and were treated bi-weekly with 1.0 mg/kg of Fabrazyme for 20 weeks. At Week 20, 12/13 (92%) patients achieved clearance to normal or near-normal levels of GL-3 accumulation in both kidney interstitial capillary and skin capillary endothelial cells ($p < 0.001$). Kidney and plasma GL-3 levels decreased by 51.9% ($p = 0.003$) and 100% ($p < 0.001$), respectively, as measured by ELISA; renal function remained normal based on serum creatinine levels and creatinine clearance. Fabry pain and quality of life were measured with the Short Form McGill Pain and SF-36 Health Survey questionnaires, respectively, and improvement over Baseline was observed in multiple categories.

These findings further confirmed the results of the larger Phase 3 Placebo-Controlled Study.

4.4 Discussion of Clinical Relevance and Summary of Results

4.4.1 Discussion of Clinical Relevance of Results

Genzyme has developed Fabrazyme containing the active ingredient recombinant human α -galactosidase A (r-h α GAL, agalsidase beta) as enzyme replacement therapy for patients with Fabry disease. We have demonstrated that Fabrazyme is taken up by cells and is transported to the lysosomes, restores the missing enzyme activity, and leads to the clearance of GL-3 from the lysosomes of various cells. We have hypothesized that prolonged treatment with Fabrazyme resulting in removal of GL-3 accumulation will lead to stabilization or possible improvement in organ function. In fact, long-term treatment with Fabrazyme in the Phase 3 Extension Study compared to corresponding, untreated historical controls suggests a slowing in the rate of progression of renal disease.

In response to FDA's questions regarding the clinical relevance of the mutually agreed upon surrogate endpoint, Genzyme has provided the following rationale.

Based on the histologic review of all kidney cell types, it was found that the GL-3 content of the critical cells involved in the pathogenesis of Fabry renal disease was significantly reduced when patients received Fabrazyme therapy at 1.0 mg/kg. Specifically,

- Fabrazyme cleared GL-3 from the endothelium of all three vessel types evaluated in the kidney (interstitial capillary endothelial cells, glomerular capillary endothelial cells and non-capillary (arteriolar) endothelial cells).
- GL-3 clearance was observed from the endothelium of cardiac and skin capillaries.

The role of endothelial and secondary vascular changes in ongoing Fabry pathology is well documented. "Normalization" resulting from Fabrazyme therapy, is indicative of at least stabilization of the disease process that would be expected to permit the kidney to retain functional reserve.

Two other cell types contribute directly to the secondary fibrotic changes that are a hallmark of severe Fabry pathology: the mesangial cells of the glomerulus (responsible for focal segmental sclerosis) and the interstitial cells of the intertubular space (a mixed population of fibroblasts and macrophage-like cells responsible for interstitial fibrosis). Both mesangial cells and interstitial cells responded to enzyme therapy with clearance of GL-3 substrate to normal or near-normal levels. This clearance of GL-3 would be expected to result in at least stabilization of the disease, if not improvement.

The vascular smooth muscle cells of arterioles and small arteries in the kidney (and in the skin – from the Phase 1/2 Study), showed a moderate but uniform reduction in GL-3 content following a period of enzyme therapy. These cells, which are critical to the control of local blood flow and ultimately responsible for maintenance of blood pressure, were heavily laden with GL-3 prior to

treatment. After approximately 12 months of enzyme therapy (6 months into the Extension study for the FZ/FZ group), the GL-3 content of vascular smooth muscle cells was considerably reduced, although not to the degree that could be characterized as “within normal limits.” This was a further improvement from that observed after 20 weeks of treatment in the Phase 3 Placebo-Controlled Study.

Of all the renal cells examined, only two cell types (podocytes and epithelial cells of the distal convoluted tubules and collecting ducts) responded with modest or inconsistent changes in GL-3 content. In both cases, despite the high load of GL-3, neither cell appears to be primarily responsible for the specific pathology that leads to failure of the Fabry kidney.

Podocytes are terminally differentiated cells integral to the glomerular filtration process through the juxtaposition of the cell’s foot processes with the basal lamina and the endothelium of the glomerular tuft capillaries. In Fabry disease, these cells, which have a very long life span, accumulate extremely high levels of GL-3 over the life of the cell starting in utero (*Tsutsumi, 1985, Asia Oceania J Obstet Gynaecol*) (*Malouf, 1976, Sci Proceedings*). As a result, minor impairment of filtration occurs from an early age with some loss of protein in the urine. These changes are well documented in female heterozygotes as well as “cardiac variants,” who have proteinuria, but these individuals do not usually develop renal failure (*Farge, 1985, Arch Pathol Lab Med*) (*Gubler, 1978, Kidney Int*) (*Marguery, 1993, Dermatology*) (*Rodriguez, 1985, Arch Pathol Lab Med*) (*Ikari, 1992, Br Heart J*) (*Junsanto, 2001, Kidney Int*) (*Elleder, 1990, Virchows Arch A Pathol Anat*). Two case studies which clearly illustrate the fact that the podocyte involvement is compatible with normal renal function have been reported by Wuthrich, et al (*Wuthrich, et al, Nephrol Dial Transplant 1998*) and Grunfeld, et al (*Grunfeld, et al, Contrib Nephrol*). Wuthrich et al described an asymptomatic 48 year old female heterozygote with normal kidney function who had a kidney biopsy performed as part of her evaluation to serve as a kidney donor. Surprisingly, the pathology revealed podocytes markedly laden with GL-3 but interstitial capillary endothelial cells that had relatively little GL-3 accumulation. Perhaps even more striking is a case report by Grunfeld et al in which the kidney from an asymptomatic female heterozygote was transplanted into a non-Fabry subject. Serial biopsies identified GL-3 inclusions in podocytes but not in other cell types. Podocyte inclusions remained unchanged for over 8 years during which time serial biopsies were performed and the transplanted kidney remained functional for 20 years. These observations indicate that, although the podocytes are significantly affected by GL-3 accumulation, involvement of these cells alone does not cause renal failure.

Genzyme concludes from the full histologic review of all relevant kidney cells harboring GL-3 inclusions, that long-term Fabrazyme therapy returned most cell types involved in renal pathophysiology to normal or near normal appearance.

In several communications, FDA has commented that the Fabry patients in the Phase 3 Placebo-Controlled Study did not exhibit clinical renal improvement. Genzyme explained that the patients had serum creatinine and other functional renal measures within the normal range at Baseline and hence, one would not expect to observe improvement in renal function. This characterization needs clarification, particularly in the context of the potential for clinical benefit from enzyme therapy. Damage by the abnormal GL-3 accumulation leads to a slow but continual loss of nephrons. It is commonly appreciated that as nephrons are lost, the remaining nephrons are put under increased stress secondary to hyperfiltration (*Hostetter, 1981, Am Physiol Soc*). When some nephrons are damaged, the remaining nephrons compensate by increasing their filtration rate (single nephron GFR or SNGFR). The net effect then is that the total GFR may remain unchanged despite substantial renal damage. The adaptive hyperfiltration per nephron reaches a maximum after about one-quarter to one-third of nephrons have been destroyed. Further loss of renal tissue is accompanied by a reduction in total GFR (*Brenner, 1982, N Engl J Med*) (*Brenner, 1983, Kidney International*) (*Olson, 1982, Kidney International*) (*Hostetter, 1981, Am Physiol Soc*).

Although not evident in terms of clinical or laboratory observations, the patients in the Phase 3 Placebo-Controlled Study all had pathologic changes to their kidneys. Benefit to patients in this situation is conserving the limited, remaining functional reserve for as long as possible. Removal of GL-3 from the cell types responsible for the relentless pathologic course of Fabry disease improves cellular function and curtails further deterioration.

4.4.2 Summary of Efficacy Results

- The results of the Phase 3 Placebo-Controlled Study showed that the histologic, primary endpoint that was prospectively agreed upon between Genzyme and FDA (clearance to normal or near-normal levels of GL-3 in the interstitial capillary endothelial cells of the kidney) was reached with a high degree of statistical significance ($p < 0.001$). This improvement was maintained during treatment with Fabrazyme for up to 12 months (the time of the last biopsy), and was confirmed in biopsies from patients who crossed-over from placebo to Fabrazyme.
- GL-3 was cleared to normal or near normal levels from other critical cell types, such as mesangial cells, glomerular capillary endothelium, interstitial cells and non-capillary endothelium, and reduced in cell types with the highest substrate burden (vascular smooth muscle cells, tubular epithelium and podocytes).
- Two of the three secondary endpoints were achieved with statistical significance:
 - composite score of GL-3 inclusions in the capillary endothelium of the kidney, skin, and heart was significantly reduced with Fabrazyme therapy ($p < 0.001$). This improvement was maintained during treatment with Fabrazyme for up to 12 months (the time of the last biopsy), and was confirmed in biopsies from patients who crossed-over from placebo to Fabrazyme.
 - Improvement was observed in rank sum score of kidney tissue and urinary GL-3 levels, as measured by ELISA. ($p < 0.003$)
- Clearance of GL-3 to normal or near-normal levels in skin biopsies has been maintained in 41/46 (89%) patients who have received Fabrazyme for 18 – 24 months. Four of the five patients that had non-zero skin scores underwent subsequent biopsies and now have zero scores. Using the Short Form McGill Pain Questionnaire, there was no significant difference between treatment groups in the clinical symptoms of pain in the Phase 3 Placebo-Controlled Study. However, there were significant improvements in multiple pain parameters within treatment groups.
- Mean serum creatinine and inulin clearance remained stable throughout the 20-week Phase 3 Placebo-Controlled Study and through 24-months of the Phase 3 Extension Study in both patient groups.
- An analysis performed to investigate differences in estimated renal events based on changes in serum creatinine for patients treated with Fabrazyme in the Phase 3 and Phase 3 Extension Studies compared to a similar, untreated historical control population showed a beneficial trend in that the point estimate for the odds ratio is approximately 0.50, corresponding to a Risk Reduction of 50% for patients treated with Fabrazyme.
- Median plasma GL-3 levels, likely a reflection of total body GL-3 accumulation, decreased by 100% in Fabrazyme-treated patients. Plasma GL-3 remains undetectable in most patients, although it has been seen to rise in patients who have missed infusions. In addition, a strong association was observed between the median percent decrease in plasma GL-3 levels and the reduction in kidney interstitial capillary endothelial cell histology scores.
- For SF-36 Health Status Survey improvements in both treatment groups were observed for several parameters across both populations, although none was statistically significant.
- Results in the 13 Japanese Fabry patients treated in the Phase 2 Open-Label Study confirmed the histologic, quality of life, and pain results of the Phase 3 Placebo-Controlled Study.

4.5 Clinical Safety

This section presents information available to date on the safety profile of Fabrazyme.

The most common adverse events (possibly, probably or definitely related per investigator assessment) are presented first organized by major clinical study as well as a subset of related events that occurred on the day of infusion (infusion-associated reactions). These data formally summarize patient exposure ranging from 5 infusions (Phase 1/2 Study) to approximately 52 infusions over approximately 2 years (Phase 3 and Phase 3 Extension Studies).

Additionally, serious adverse events are organized by summary listings of patient deaths, 4 major clinical studies and all other on-going studies, compassionate use/special access and spontaneous reports from patients receiving commercial product in the 23 countries in which Fabrazyme is already approved. These data summarize all reported serious adverse events through 01 July 2002 reflecting a maximum possible patient exposure of approximately 3 years of Fabrazyme treatment.

Full Safety Data - Completed Studies

- Phase 1/2 Study
- Phase 3 Placebo-Controlled Study
- Phase 2 Open-Label Study (Japan)

Full Safety Data - Ongoing Studies (18 – 24 months Fabrazyme treatment)

- Phase 3 Extension Study

Serious Adverse Events Only - Ongoing Studies/Programs

- Phase 4 Double-Blind, Placebo-Controlled Study
- Phase 1/2 Extension Study
- Phase 2 Extension Study (Japan)
- United States Single Patient Exemption
- Compassionate Use/ Special Access Programs – Canada; Australia; Japan; European Union; France ATU (Authorization Temporaire d'Utilisation)
- Physician Sponsored Study - Dr. G. Linthorst (The Netherlands) Fabrazyme vs. Replagal™ 0.2mg/kg
- Post-Approval Spontaneous Reports

In this report, duration of treatment for patients in different studies is summarized for the four major studies: Phase 1/2 Open-Label, Phase 3 Placebo-Controlled, Phase 3 Extension and Phase 2 Open-Label (Japan). Table 4-13 summarizes patient exposure to Fabrazyme for each study.

Table 4-13 Number of Patients and Exposure to Fabrazyme for the Four Major Studies

STUDY	TREATMENT / DOSE GROUP	Exposure to Fabrazyme							
		10 Days	10 weeks	20 weeks	6 months	1 year	18 months	2 years	30 months ^A
Phase 1/2 Study	0.3 mg/kg		3						
	1.0 mg/kg		3						
	1.0 mg/kg	3							
	3.0 mg/kg		3						
	3.0 mg/kg	3							
Phase 3 Placebo-Controlled	Placebo			0					
	Fabrazyme			29					
Phase 3 Extension*	Placebo/Fabrazyme				28	26	27	25	
	Fabrazyme/Fabrazyme				29	29	26	27	23
Phase 2 Open-Label (Japan)	1.0 mg/kg		13						

* Patients from the Phase 3 Placebo-Controlled Study also participate in the Phase 3 Extension Study. Fluctuations in the patient numbers between different time points are due to missed infusions at those particular time points.

^A Formal patient exposure information available through 30 months only; longest patient on therapy ~ 3 years.

We estimate that over 350 patients have received over 4000 total infusions of Fabrazyme (including patient experience with Fabrazyme through compassionate use/special access, other on-going studies and post-approval) with the longest patient on therapy for over 3 years.

4.5.1 Adverse Events

4.5.1.1 Most Common

Due to the differing study designs, the most common adverse events for the four major studies are discussed in the context of each individual study. In order to evaluate adverse events that were most likely related to the administration of Fabrazyme, related adverse events that occurred on the day of infusion were further subsetted and an additional analysis was performed. These adverse events are referred to as infusion-associated reactions (IAR) (see Section 4.5.1.2).. Laboratory data from blood samples collected prior to Fabrazyme infusions have been analyzed separately.

Phase 1/2 Study

A Phase 1/2, dose-ranging, open-label study in 15 patients with Fabry disease was conducted to evaluate the safety and pharmacokinetics of Fabrazyme in Fabry patients. The study was also designed to obtain dose response information and identify an effective dose for future study.

There were five treatment groups consisting of 3 patients each: 0.3 mg/kg every 14 days; 1.0 mg/kg every 48 hours; 1.0 mg/kg every 14 days; 3.0 mg/kg every 48 hours; and 3.0 mg/kg every 14 days. The overall conclusion was Fabrazyme therapy follows a relative dose response in clearing glycosphingolipid. No formal-hypothesis testing was performed due to the small sample size in each treatment group (n=3).

The most common adverse event from patients receiving Fabrazyme (all doses) consisted of mild to moderate transient elevations in blood pressure, reported for patients in all five dosing groups. A review of all blood pressure measurements taken at each of the 5 Fabrazyme infusions (for all 15 patients) revealed that the mean of the maximum systolic change from baseline was approximately 10 mmHg.

Other common adverse events reported by the investigator included "allergic reaction" (symptoms described as nausea, vomiting, diaphoresis, urticaria, edema, pruritus, bradycardia, abdominal pain), pain, headache, fever, or abdominal pain. The majority of adverse events were mild in severity and were attributed as either possibly or probably related to study drug administration. Two patients experienced serious adverse events (see Section 4.5.2); no patients discontinued from the study due to an adverse event.

Phase 3 Placebo-Controlled Study

Fifty-eight patients enrolled in the Phase 3 randomized, double blind, placebo-controlled study and were randomized to receive active (Fabrazyme) at 1.0 mg/kg every 2 weeks or placebo treatment. The infusion rate for all patients was 0.25 mg/min which helped to maintain the blind.

Postoperative pain, which captures pain related to a protocol-specified biopsy procedure, was the most frequently reported adverse experience in both the Fabrazyme (76%) and the placebo (55%) treatment groups. (Table 4-14)

A statistically significant difference was observed for three adverse events that were reported more frequently in patients treated with Fabrazyme compared to placebo patients. These adverse experiences included rigors (15/29 [52%] vs. 4/29 [14%], $p=0.004$), fever (14/29 [48%] vs. 5/29 [17%], $p=0.024$), and skeletal pain (6/29 [21%] vs. 0/29 [0%], $p=0.023$) in the Fabrazyme and placebo treatment groups, respectively. No adverse event occurred significantly more frequently in the placebo group compared to the Fabrazyme group.

The preferred term "rigors" captured such verbatim terms as chills, shaking chills, and cold flashes. The majority of these events were associated with Fabrazyme infusions. Six of the 15 patients in the Fabrazyme group who experienced rigors also had a concurrent report of fever during one or more infusion-associated reactions. For the placebo patients who reported rigors: one patient experienced rigors on the day of a renal biopsy; one patient experienced chills and fever as part of a symptom complex suggestive of flu-like symptoms; one patient experienced

chills prior to infusion; and one patient experienced chills which occurred six hours after the infusion was completed.

Each of the 14 individual patient adverse event reports of fever in the Fabrazyme group were reviewed to determine if they were associated with an infusion of Fabrazyme. Eight of the 14 patients were confirmed to have had an episode of fever reported on a day other than the scheduled day of infusion. Therefore, these eight reports are not considered to be infusion-related fevers, and the remaining 6 were considered infusion-associated reactions.

There were no reports of fever that occurred on the day of the infusion among placebo treated patients.

Skeletal pain also was reported only in the Fabrazyme treatment group and presented single episodes primarily as neck and shoulder pain/discomfort, which were mild to moderate in severity but did not occur on the day of infusion. None of these reports was considered related to treatment.

In summary, two statistically significant terms, rigors and fever, primarily represent infusion-associated reactions. The initial presentation of these most often coincided with IgG seroconversion (see Section 4.5.1.2). When rigors and fever occurred, they were generally successfully managed by a temporary reduction in infusion rate and treatment with acetaminophen and diphenhydramine.

Many Fabry patients have evidence of musculoskeletal involvement. Skeletal pain reported during this study represents isolated musculoskeletal events and is most likely not due to the infusion of Fabrazyme.

Phase 3 Extension Study

Adverse events are reported through Infusion 41, representing approximately 18 – 24 months of open-label treatment with Fabrazyme. Table 4-15 represents adverse events by decreasing order of overall frequency and by relationship to treatment with Fabrazyme.

The most common adverse events without regard to relationship to treatment include rhinitis, rigors, fever and headache. The most common related adverse events are rigors, temperature changed sensation (“feels cold”), fever and headache. Albuminuria represents a laboratory adverse event that primarily captures an increase in urinary protein. The majority of reports of albuminuria are not considered related to treatment with Fabrazyme. Most reported adverse events are mild to moderate in severity.

Table 4-14 Phase 3 Placebo-Controlled Study: Adverse Events that Occurred in at Least 10% of Patients (in Fabrazyme Treatment Group) by Relatedness

TREATMENT GROUP (as treated)	Placebo (n = 29)		Fabrazyme (n = 29)	
	Not Related n (%)	Related n (%)	Not Related n (%)	Related n (%)
ADVERSE EVENT*				
Postoperative Pain	16 (55)	0	22 (76)	0
Rigors**	4 (14)	0	1 (3)	14 (48)
Fever**	4 (14)	1 (3)	7 (24)	7 (24)
Headache	9 (31)	2 (7)	8 (28)	5 (17)
Rhinitis	7 (24)	0	9 (31)	2 (7)
Hematuria	7 (24)	0	8 (28)	2 (7)
Abdominal Pain	8 (28)	1 (3)	7 (24)	1 (3)
Anxiety	5 (17)	0	8 (28)	0
Nausea	4 (14)	0	6 (21)	2 (7)
Pharyngitis	2 (7)	0	8 (28)	0
Anemia	7 (24)	3 (10)	5 (17)	2 (7)
Coughing	6 (21)	0	7 (24)	0
Pain	3 (10)	0	6 (21)	0
Edema Dependent (in extremities)	1 (3)	0	5 (17)	1 (3)
Skeletal Pain**	0	0	6 (21)	0
Fabry Pain	2 (7)	1 (3)	3 (10)	3 (10)
Renal Function Abnormal	9 (31)	0	6 (21)	0
Chest Pain	3 (10)	0	4 (14)	1 (3)
Fatigue	2 (7)	4 (14)	4 (14)	1 (3)
Temp. Changed Sensation (feels cold)	1 (3)	0	1 (3)	4 (14)
Heart Valve Disorders	4 (14)	0	5 (17)	0
Vomiting	4 (14)	0	3 (10)	2 (7)
Asthenia	3 (10)	1 (3)	4 (14)	0
Pallor	1 (3)	0	2 (7)	2 (7)
Hypotension	2 (7)	0	3 (10)	1 (3)
Dizziness	1 (3)	1 (3)	3 (10)	1 (3)
Paraesthesia	2 (7)	0	2 (7)	2 (7)
Cardiomegaly	1 (3)	0	3 (10)	0
Hypertension	0	0	0	3 (10)
Dyspepsia	1 (3)	0	3 (10)	0
Bradycardia	7 (24)	0	3 (10)	0
Arthrosis	0	0	2 (7)	1 (3)
Myalgia	4 (14)	0	1 (3)	2 (7)
Depression	1 (3)	0	3 (10)	0
Insomnia	2 (7)	0	3 (10)	0
Bronchitis	1 (3)	0	3 (10)	0
Dyspnea	2 (7)	0	2 (7)	1 (3)
Pruritus	2 (7)	1 (3)	2 (7)	1 (3)
Albuminuria	3 (10)	0	3 (10)	0
Eye Abnormality	1 (3)	0	3 (10)	0
Vision Abnormal	1 (3)	1 (3)	2 (7)	1 (3)

* WHOART Preferred Term (modified WHOART Dictionary, Q2 1993).

** p<0.05, Fisher's Exact Test

Table 4-15 Phase 3 Extension Study: Adverse Events in at Least 10% of Patients (Overall) as of the 18 Month Timepoint of Phase 3 Extension Study

TREATMENT GROUP (as treated) <i>Fabrazyme Exposure</i>	Placebo/Fabrazyme (n = 29)		Fabrazyme/Fabrazyme (n = 29)	
	~18 months		~24 months	
ADVERSE EVENT*	Not Related n (%)	Related n (%)	Not Related n (%)	Related n (%)
Rhinitis	12 (41)	7 (24)	21 (72)	1 (3)
Rigors	2 (7)	16 (55)	1 (3)	15 (52)
Albuminuria	14 (48)	1 (3)	11 (38)	2 (7)
Fever	8 (28)	7 (24)	5 (17)	7 (24)
Headache	4 (14)	6 (21)	11 (38)	6 (21)
Coughing	8 (28)	1 (3)	14 (48)	1 (3)
Renal Function Abnormal	12 (41)	2 (7)	7 (24)	2 (7)
Pain	10 (34)	3 (10)	6 (21)	3 (10)
Temp. Changed Sensation ("feels cold")	2 (7)	11 (38)	0	9 (31)
Heart Valve Disorders	7 (24)	3 (10)	9 (31)	2 (7)
Vomiting	4 (14)	3 (10)	11 (38)	3 (10)
Nausea	2 (7)	4 (14)	4 (14)	9 (31)
Cardiomegaly	6 (21)	1 (3)	10 (34)	1 (3)
Pharyngitis	9 (31)	0	9 (31)	0
Upper Respiratory Tract Infection	9 (31)	0	9 (31)	0
Anemia	5 (17)	3 (10)	7 (24)	2 (7)
Post-Operative Pain	7 (24)	1 (3)	9 (31)	0
Chest Pain	4 (14)	7 (24)	3 (10)	2 (7)
Edema Dependent (in extremities)	6 (21)	3 (10)	5 (17)	2 (7)
Abdominal Pain	5 (17)	2 (7)	4 (14)	4 (14)
Fabry Pain	3 (10)	6 (21)	4 (14)	2 (7)
Influenza-Like Symptoms	8 (28)	0	6 (21)	1 (3)
Paraesthesia	4 (14)	1 (3)	6 (21)	3 (10)
Back Pain	4 (14)	2 (7)	6 (21)	1 (3)
Myalgia	3 (10)	3 (10)	2 (7)	5 (17)
Fatigue	3 (10)	4 (14)	4 (14)	1 (3)
Heart Disorder	4 (14)	4 (14)	2 (7)	2 (7)
Asthenia	3 (10)	2 (7)	4 (14)	2 (7)
Bradycardia	4 (14)	2 (7)	3 (10)	2 (7)
Bronchitis	4 (14)	0	7 (24)	0
Dyspnea	2 (7)	4 (14)	2 (7)	3 (10)
Pruritus	2 (7)	4 (14)	2 (7)	3 (10)
Diarrhea	6 (21)	0	3 (10)	1 (3)
Dizziness	4 (14)	0	3 (10)	3 (10)
ECG Abnormal	2 (7)	1 (3)	5 (17)	2 (7)
Somnolence ^A	0	5 (17)	2 (7)	3 (10)
Anxiety	6 (21)	0	2 (7)	1 (3)
Depression	3 (10)	0	6 (21)	0
Dyspepsia	5 (17)	1 (3)	3 (10)	0
Heart Block	4 (14)	0	4 (14)	1 (3)
Bronchospasm	2 (7)	2 (7)	2 (7)	2 (7)
Flushing	1 (3)	6 (21)	0	1 (3)
Retinal Disorder	3 (10)	1 (3)	4 (14)	0
Skeletal Pain	3 (10)	1 (3)	4 (14)	0
Vision Abnormal	1 (3)	2 (7)	3 (10)	2 (7)
Cardiac Failure	1 (3)	3 (10)	1 (3)	2 (7)
Haematuria	3 (10)	0	4 (14)	0
Hypertension	2 (7)	1 (3)	1 (3)	3 (10)
Malaise	3 (10)	1 (3)	2 (7)	1 (3)
Rash	1 (3)	1 (3)	4 (14)	1 (3)
Acne	2 (7)	1 (3)	2 (7)	1 (3)
Bundle Branch Block	3 (10)	0	2 (7)	1 (3)
Hearing Decreased	3 (10)	0	3 (10)	0
Infection	4 (14)	1 (3)	1 (3)	0
Injury Accident	3 (10)	0	3 (10)	0
Leg Pain	0	3 (10)	1 (3)	2 (7)
Palpitation	2 (7)	2 (7)	1 (3)	1 (3)
Tremor	1 (3)	3 (10)	0	2 (7)

* Modified WHOART Dictionary (Q2 1993).

^A Somnolence was primarily attributed to pre-infusion treatment with antihistamines.

Phase 2 Open-Label Study (Japan)

The adverse events considered related to treatment experienced by Japanese patients participating in the Phase 2 Open-Label Study (Japan) were similar to those reported by the Fabrazyme treatment groups of the Phase 3 Placebo-Controlled Study and the Phase 3 Extension Study. All three studies utilized the same dose of 1.0 mg/kg every 2 weeks. The majority of these events were considered mild in intensity and consisted primarily of rigors, fever, headache, and rhinitis.

4.5.1.2 Infusion-Associated Reactions (IAR)

It is well established that immune responses may occur in patients infused with recombinant proteins. Related adverse events that occurred on the day of infusion were further subsetted, analyzed and grouped as infusion-associated reactions (IAR) in order to evaluate adverse events that were most likely related to the administration of Fabrazyme. The strategy for antibody testing and management of these types of reactions was outlined in each study protocol.

The influence of IgG-mediated immune responses during or immediately after the reaction may be the likely cause of a number of the observed symptoms including fevers and chills that were observed with Fabrazyme infusions.

During the Phase 1/2 Open-Label Study, four patients developed symptoms that were initially suspected to be immune-mediated during one or more infusions with Fabrazyme. Three of these four patients received 3.0 mg/kg every 14 days (dose Group 3) compared to one patient who received 1.0 mg/kg every 14 days (dose Group 2) and none who received 0.3 mg/kg every 14 days (dose Group 1). All four patients tested positive for IgG antibodies. Three of 4 patients continue to receive Fabrazyme (1.0 mg/kg every 2 weeks) in the Phase 1/2 Extension Study. The fourth patient continues to receive Fabrazyme (1.0 mg/kg every 2 weeks) through Compassionate Use in Australia.

During the Phase 3 Placebo-Controlled Study, 19 out of 29 active treatment patients (66%) experienced infusion-associated reactions compared to 8/29 placebo patients. Sixteen of these 19 patients that received active treatment were found to have developed IgG antibodies. Serologic analyses for circulating immune complexes conducted in the Fabrazyme-treated patients tested negative. (See Section 4.5.3.3, Immune Complexes) The most commonly reported symptoms for Fabrazyme-treated patients were chills (rigors) and increased temperature (fever). Other reported symptoms included, but were not limited to extremity pain, cold feelings, headaches and vomiting. These reactions were generally transient, mild or moderate in severity, and were not serious or life threatening.

Patients in the Phase 3 Extension Study have received 18 months (placebo/Fabrazyme group) to 24 months (Fabrazyme/ Fabrazyme group) of Fabrazyme treatment. The analysis of individual adverse event terms has not revealed any unexpected infusion-associated reactions not previously

identified. Rigors, fever, temperature changed sensation, headache and nausea are the most common related adverse events experienced by patients on the day of infusion who have received Fabrazyme the longest period of time.

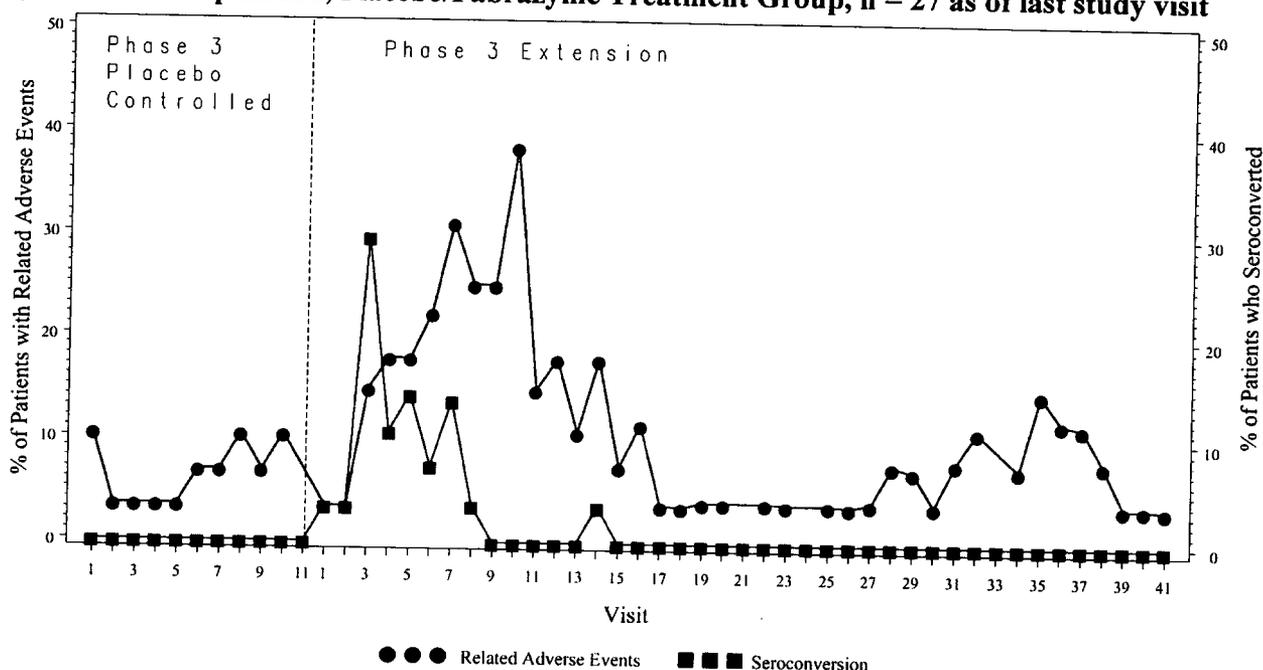
The data demonstrate that the total number of patients experiencing any infusion-associated reaction has decreased over time when compared to the Phase 3 Placebo-Controlled Study and across discrete time points during the Phase 3 Extension Study. (Table 4-16) The majority of patients that have experienced IARs at later time points (6-12 months and 12-18 months in the Phase 3 Extension Study) are the same patients that have experienced IARs at the earlier time-points (Phase 3 Placebo-Controlled Study: Baseline – week 20 and Phase 3 Extension Study: Entry – 6 month). In addition, at both the 6-12 months and 12-18 months time periods in the Phase 3 Extension Study, there were no more than 2 unique patients from either the placebo/Fabrazyme or Fabrazyme/Fabrazyme treatment groups who experienced new IARs (i.e., who did not experience IARs at any of the earlier time points). In addition, the unique patients in the Phase 3 Extension Study that experienced IARs during the 6-12 months time period did not experience IARs during the 12-18 months period. The reduction in IARs observed over the 18-24 month treatment period is likely a function of multiple factors, including administration experience, pre-treatment and rate adjustments based on individual patient responses, and a reduction in IgG antibody titers. Additionally, improvements in endothelial cell function as a result of Fabrazyme treatment may lead to reduced inflammatory cytokine release. Collectively, the data suggest that there is an overall reduction in IARs, and that during long-term treatment with Fabrazyme one would expect the safety profile to remain favorable without an increase in the number of adverse events reported over time.

Table 4-16 Number of Patients Experiencing Infusion-Associated Reactions

TREATMENT GROUP	Phase 3 Placebo Controlled		Phase 3 Extension						
	Placebo	Fabrazyme	Placebo/ Fabrazyme	Fabrazyme/ Fabrazyme		Fabrazyme/ Fabrazyme		Placebo/ Fabrazyme	Fabrazyme/ Fabrazyme
				Placebo/ Fabrazyme	Fabrazyme/ Fabrazyme	Placebo/ Fabrazyme	Fabrazyme/ Fabrazyme		
<i>Exposure to Fabrazyme (months)</i>	<i>0</i>	<i>5</i>	<i>6</i>	<i>12</i>	<i>12</i>	<i>18</i>	<i>18</i>	<i>24</i>	
Total Patients with Infusion-Associated Reactions	8	19	18	16	12	14	9	6	
Rigors	0	14	14	12	2	8	4	2	
Somnolence	4	2	3	1	5	3	1	0	
Temperature Change Sensation	0	3	10	8	1	5	1	0	
Fever	0	7	5	5	1	3	2	0	
Rhinitis	0	2	6	1	1	0	1	0	
Nausea	0	2	3	4	1	2	0	4	

Figure 4-18 illustrates the association between the percent of patients with related adverse events and the percent of patients who seroconverted at each infusion in the Placebo/Fabrazyme treatment group. The majority of patients seroconvert between Infusions 3 and 7 of Fabrazyme treatment. The frequency of related adverse events on the day of infusion increases in parallel to the increase in seroconversion and then subsequently decreases over time.

Figure 4-18 Percent of Patients with Related Adverse Events (Excluding Somnolence) on the Same Day as Infusion and Percent of Patients who Seroconverted at Each Visit (As Treated Population, Placebo/Fabrazyme Treatment Group, n = 27 as of last study visit)



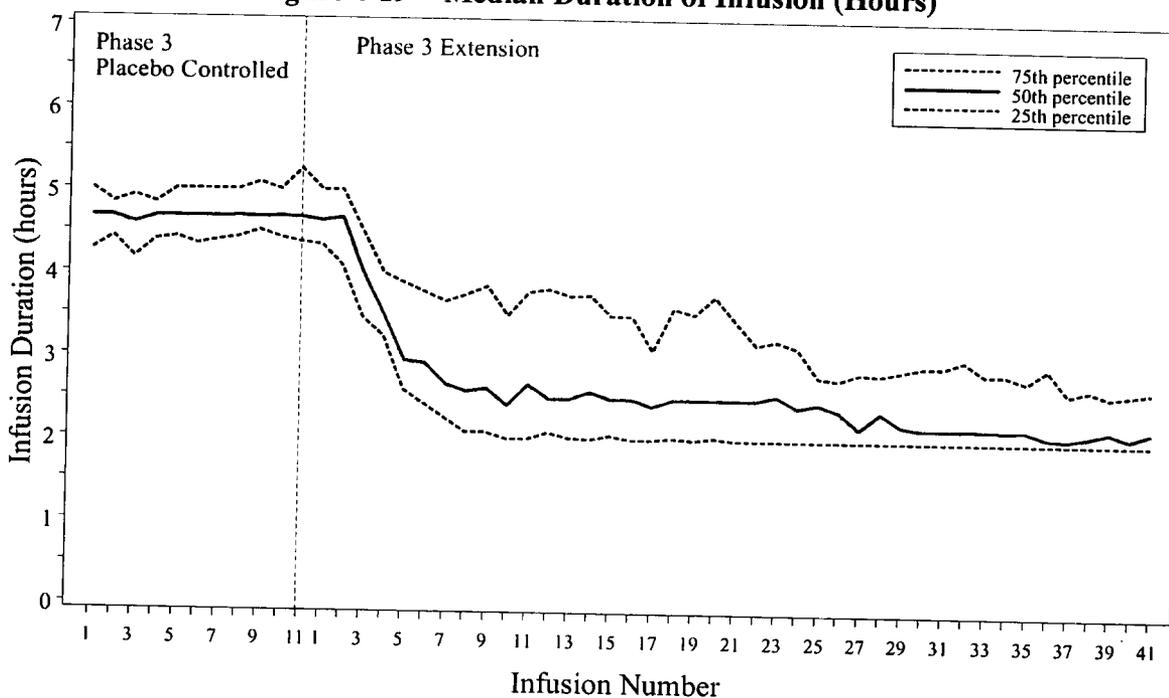
During the Phase 2 Open-Label Study (Japan), 10 of the 13 Japanese patients experienced infusion-associated reactions (excluding one report of dry skin on the day of infusion) and 8 of the 10 patients developed IgG antibodies. The most commonly reported symptoms were chills (rigors) and fever. Other reported symptoms included, but were not limited to, rhinitis, dyspnea, hypertension, and malaise.

Across the three primary studies using the 1.0 mg/kg every two weeks Fabrazyme dosing regimen, Phase 3 Placeto-Controlled Study, Phase 3 Extension Study, and Phase 2 Open-Label (Japan), a single distinct pattern was observed. The majority of initial symptom reports appeared between infusion numbers 4 – 8 most often initially occurred at the time of seroconversion (see Section 4.5.3), about one hour into the infusion. They were generally mild to moderate in nature, associated with infusions and usually managed with conservative measures. The initial management of these patients included the administration of a single dose of an antihistamine and/or an antipyretic (and occasionally oral steroids and/or inhaled β -agonist) accompanied by a reduction in the infusion rate by 1/4 to 1/2 of the original

rate, until the episode abated. The infusion usually was either completed at a reduced rate or gradually titrated up to the initial rate. The number of patients who experienced infusion-associated reactions has decreased over time.

In addition, long-term data has demonstrated patients are able to successfully tolerate an increase in infusion rate resulting in shortened total infusion time. As of the 18 month timepoint of the Phase 3 Extension Study, 50/58 (86%) patients completed one or more complete infusions of Fabrazyme (1.0 mg/kg) with an infusion time of ≤ 2.5 hours. Further, as of the 18 month timepoint of the Phase 3 Extension Study, 35/58 (60%) patients completed one or more complete infusions of Fabrazyme (1.0 mg/kg) with an infusion time of ≤ 2.0 hours. (Figure 4-19)

Figure 4-19 Median Duration of Infusion (Hours)



4.5.2 Serious Adverse Events

4.5.2.1 Deaths

This section lists all patient deaths that occurred in clinical studies that have been completed, as well as any deaths that have occurred up through 01 July 2002 during ongoing extension, compassionate use studies, special access programs or spontaneous post-approval reports.

No deaths were reported in the Phase 1/2 Study, Phase 3 Placebo-Controlled Study, and Phase 2 Open-Label (Japan) Study. One death has been reported in the Phase 3 Extension Study. This patient died of cardiac arrest. The investigator reported the death as “possibly related” to

Fabrazyme therapy with the rationale that clearance of GL-3 from cardiac tissue may have contributed to a dysrhythmia.

Eight deaths occurred before 01 July 2002 in other ongoing extension, compassionate use studies/special access programs, French Authorisation Temporaire d'Utilisation (ATU), Phase 4 Double-Blind, Placebo-Controlled Study or spontaneous post-approval reports. None of these eight deaths was considered to be related to treatment with study drug as reported by the investigator. (Table 4-17)

Table 4-17 Summary of All Death Reports through 01 July 2002

Source/ Patient ID	Age/ Sex	Days of Fabrazyme treatment prior to Death	Description	Relationship to Fabrazyme	Medical History
Phase 3 Extension/0506	43/M	400	Cardiac arrest, Dysrhythmia	Possible	Severe heart disease associated with marked acute heart failure and findings consistent w/ Fabry disease
Phase 4*/18041	51/M	25 (blinded)	Cardiac arrest	Unlikely	Hyperlipidemia, gout, and kidney stones and findings consistent w/ Fabry disease
Phase 4*/12041	55/M	2 (blinded)	Stroke	Not Related	Ischaemic heart disease, decreased left ventricular function, renal impairment
US Single Patient Exemption / CAC	59/M	1	Anasarca; Sepsis	Not Related	Severe Fabry disease; ESRD; Restrictive lung disease; Severe restrictive cardiomyopathy; Chronic atrial fibrillation; Severe ventricular cavity obliteration; Pericardial effusion; Bilateral pleural effusion; CVA with mild left hemiparesis.
Europe CU (Netherlands)	48/M	5	Cardiac Arrest	Unlikely	Four myocardial infarctions (1986, 1989, 1997, 1998).
Japan CU	63/M	263	Ventricular Tachycardia	Not Related	Extensive cardiac history including sick sinus syndrome, congestive heart failure, left ventricular hypertrophy
Europe CU (French ATU)	53/M	42	Ischemic colitis, Multi-organ failure	Unlikely	Haemodialysis 3 times a week, long history of abdominal pain
Post-approval	46/M	306	Cardiac Arrest	Not Related	Congenital heart abnormality; COPD; chronic kidney insufficiency
Post-approval	42/M	424	Brain Stem Infarct	Not Related	Kidney Transplant

*Treatment assignments have not been unblinded for the Phase 4 Placebo-Controlled Study.

4.5.2.2 Other Serious Adverse Events (SAE)

During the Phase 1/2 Study, two patients experienced serious adverse events. These events occurred in patients in the 1.0 mg/kg every 14 days and 3.0 mg/kg every 48 hours treatment groups respectively. Both patients continue to receive Fabrazyme in the Phase 1/2 Extension Study (1.0 mg/kg every 2 weeks) at a current rate of 250 ml/hr. (Table 4-18)

Table 4-18 Phase 1/2 Study Serious Adverse Events

Patient	Age/ Sex	Treatment Group/ Dose	Days of Fabrazyme treatment prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
5	36/M	Fabrazyme 1.0 mg/kg q14 days	40	Allergic Reaction	Probable	Infusion-associated reactions during 2 previous Fabrazyme infusions.
14	30/M	Fabrazyme 3.0mg/kg q48 hrs	12	Pulmonary Emboli	Possible	History of deep vein thrombosis (DVT) maintained with warfarin; warfarin stopped at study enrollment.

During the Phase 3 Placebo-Controlled Study, 10 patients (five in each treatment group) had serious adverse event reports (SAE). None of the SAEs that occurred in either treatment group was considered related to study medication by the reporting physician.

The most significant observation regarding SAEs that occurred during the study is that most of the SAEs reported in both treatment groups were associated with the protocol-required biopsy procedures. (Table 4-19) All of the other SAEs in both treatment groups were associated with documented underlying disease or accidents. (Table 4-20)

**Table 4-19 Phase 3 Placebo-Controlled Study:
Serious Adverse Events in Order of Frequency**

	Placebo	Fabrazyme
Total number of patients	29	29
Number of Patients with SAEs	5	5
Total Number of SAEs	14	5
Event		
Hemopericardium	1	1
Angina pectoris	1	0
Cellulitis	0	1
Chest pain	0	1
Convulsions	1	0
Depression	0	1
Hematoma	1	0
Hematuria	1	0
Hemorrhage intracranial	1	0
Hemorrhage NOS	1	0
Hypotension	0	1
Injury accident	1	0
Paraesthesia	1	0
Pericardial effusion	1	0
Post-Operative pain	1	0
Speech disorder	1	0
Syncope	1	0
Thrombosis coronary	1	0

Table 4-20 Phase 3 Placebo-Controlled Study: Summary of Serious Adverse Events

Patient	Age/ Sex	Treatment Group	Days of study drug treatment prior to SAE	SAE Description	Relationship to Study Medication	Medical History
115	21/M	Fabrazyme	157	Decreased Blood Pressure	Not Related	Cardiac biopsy
305	37/M	Fabrazyme	30	Cellulitis	Not Related	Chronic osteomyelitis
602	44/F	Fabrazyme	151	Chest Pain	Not Related	Cardiac biopsy
605	19/F	Fabrazyme	157	Depression Worsened	Not Related	History of depression
801	33/M	Fabrazyme	143	Cardiac Perforation	Not Related	Cardiac biopsy
111	40/M	Placebo	152	Vaso-Vagal Response	Not Related	Renal biopsy
303	34/M	Placebo	117	Cortical Contusion	Not Related	Fall; head trauma
			117	Slurred Speech	Not Related	
			118	Seizures	Not Related	
502	61/M	Placebo	93	Worsening of Angina	Not Related	Angina/coronary artery disease, Coronary artery bypass graft surgery
			93	Coronary Artery Occlusion	Not Related	
			94	Surgical Bleed	Not Related	
			105	Pericardial Effusion	Not Related	
703	27/M	Placebo	141	Paraesthesia	Not Related	Fabry disease
			141	Abdominal Pain	Not Related	Kidney biopsy
803	28/M	Placebo	-7 ^A	Cardiac Perforation	Not Related	Baseline cardiac biopsy
			-5 ^A	Hematuria	Not Related	Baseline kidney biopsy
			-5 ^A	Retroperitoneal Hematoma	Not Related	
			15	Subdural Hematoma	Unlikely	Excessive alcohol consumption, Fall

A - ^A indicates the SAE occurred before the first infusion

As of the 18 month timepoint of the Phase 3 Extension Study, 20 patients had serious adverse event reports (SAE). Table 4-21 summarizes the serious adverse events in order of frequency and reported relatedness to Fabrazyme.

Table 4-21 Phase 3 Extension Study: Summary of Serious Adverse Events in Order of Frequency and Stratified by Relatedness as of the 18 Month Timepoint

Total Number of Patients		58	
Number of Patients with SAEs		20/58 (34%)	
Total Number of SAEs		43	
Event	Not Related n (%)	Related n (%)	
Chest pain	3 (5)	1 (2)	
Injury accident	2 (3)	0	
Tachycardia	0	2 (3)	
Angina pectoris	1 (2)	0	
Aphasia	0	1 (2)	
Arrhythmia	0	1 (2)	
Basal cell carcinoma	1 (2)	0	
Bone disorder	1 (2)	0	
Bradycardia	0	1 (2)	
Bronchitis	1 (2)	0	
Cardiac arrest	0	1 (2)	
Cardiac failure	0	1 (2)	
Carpal tunnel syndrome	1 (2)	0	
Cellulitis	1 (2)	0	
Cerebrovascular disorder	0	1 (2)	
Fever	0	1 (2)	
Hematuria	1 (2)	0	
Hemorrhage NOS	1 (2)	0	
Hearing decreased	1 (2)	0	
Heart murmur	1 (2)	0	
Herpes simplex	1 (2)	0	
Hypertension	0	1 (2)	
Hypotension	1 (2)	0	
Macular edema	1 (2)	0	
Nephrosis*	0	1 (2)	
Paralysis	0	1 (2)	
Pericardial effusion	1 (2)	0	
Pericarditis	1 (2)	0	
Peripheral ischaemia	1 (2)	0	

* The relationship of Fabrazyme for the report of nephrosis was changed by the investigator to unlikely related upon follow-up.

When expanded beyond the Phase 3 Extension Study formal listings (as of the 18 month timepoint), 3 additional patients have experienced SAEs through 01 July 2002. Table 4-22 summarizes all serious adverse events through 01 July 2002.

Table 4-22 Phase 3 Extension Study: Serious Adverse Events through 01 July 2002

Patient	Age/ Sex	Treatment Group ⁺	Days of Fabrazyme treatment prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
108	51/M	FZ/FZ	981	Viral Pericarditis	Not Related	No related medical history
109	37/M	FZ/FZ	392	Haematuria	Not Related	Related to kidney biopsy.
			690	Hand Injury	Not Related	No related medical history
			770	Stroke	Not Related	No related medical history
112	25/M	FZ/FZ	870	Stroke	Not Related	No related medical history
115	21/M	FZ/FZ	240	Attempted Suicide	Unlikely	No related medical history
			846	Drug Overdose (cocaine)	Unlikely	2 previous suicide attempts
116	26/M	PL/FZ	908	Head injury, Hand injury	Not Related	No related medical history
201	21/M	PL/FZ	32	Tachycardia,	Definite	Infusion-associated reaction
			33	Hypertension		
202	47/M	FZ/FZ	371	Decreased Blood Pressure	Not Related	Related to kidney biopsy.
			371	Hemorrhage	Not Related	Related to kidney biopsy.
304	23/M	PL/FZ	98	Pruritic Urticaria	Definite	Infusion-associated reaction
306	20/M	PL/FZ	55	Tightness In Chest and Throat	Definite	Infusion-associated reaction
307	31/M	FZ/FZ	604	Bronchitis	Not Related	Asthma, tobacco use
501	42/M	FZ/FZ	611	Stroke	Possible	Multiple strokes, residual dysphasia, weakness
				Worsening Angina	Not Related	Angina, dyspnea, atrial fibrillation, & systolic heart murmur.
506	43/M	PL/FZ	242	Bradycardia, Decreased Cardiac Output	Possible	Severe heart disease associated with marked acute heart failure and findings consistent with history of Fabry disease
			400	Cardiac Arrest , Dysrhythmia (Section, 4.5.2.1 Deaths)	Possible	
603	20/M	PL/FZ	35	Chest Pain	Not Related	No known cardiac history
605	20/F	FZ/FZ	414	Pericardial Chest Pain	Not Related	Related to cardiac biopsy.
			414	Pericardial Effusion	Not Related	
			414	Pericardial Rub	Not Related	
			414	Pericarditis	Not Related	
703	27/M	PL/FZ	72	Vertigo	Not Related	Vestibulocochlear disorders, tinnitus, bilateral hearing loss, vertigo
			79	Hypoacusia	Not Related	
			231	Vertigo	Not Related	
705	23/M 24/M	FZ/FZ	622	Carpal Tunnel Syndrome	Not Related	Infusion-associated reaction
			881	Swelling Right Ear ^a	Definite	
			881	Erythema Right Ear ^a	Definite	
			881	Warmth Sensation in Face ^a	Definite	
706	43/M	FZ/FZ	584	Herpes Labialis	Not Related	No relevant medical history
			584	Erysipelas right leg	Not Related	
708	41/M	PL/FZ	9	Thoracic Pain with Oppression	Not Related	Related to cardiac biopsy.
			-12	Pericardial Effusion	Not Related	
			175	Transitory Ischemia (Hand)	Not Related	
803	31/M	PL/FZ	695	Suicide Attempt	Not Related	No relevant medical history
			730	Chest Pain	Not Related	
804	43/M 44/M	FZ/FZ	254	Basal Cell Carcinoma (cheek)	Not Related	No relevant medical history
			625	Nephrotic Syndrome	Unlikely	History of Fabry-related proteinuria.
			923	Nephrotic Syndrome Worsened	Unlikely	Extensive baseline glomeruli scarring
805	18/M	PL/FZ	66	Loss Of Visual Acuity	Unlikely	History of inflammatory syndrome
			66	Macular Oedema	Unlikely	
			66	Retinal White Dots Syndrome	Unlikely	
			128	Fever, Very Intense Shivering, Tachycardia	Probable	Infusion-associated reaction
806	30/M	FZ/FZ	539	First Metatarsal Osteitis	Not Related	History cutaneous wound ulcer
			582	Abdominal Pain, Cutaneous Rash, Skin Redness, Vomiting, Pruritus, Nausea, Shivering	Definite	Likely infusion-associated reaction

⁺ - PL/FZ = Placebo/Fabrazyme treatment group ; FZ/FZ = Fabrazyme/Fabrazyme treatment group
^A - These events were reported as non-serious, but were considered as important medical events

Serious adverse events considered related to treatment with Fabrazyme consisted of infusion-associated symptoms such as tachycardia, fever, hypertension or cardiac events. There was one report of CVA in a patient with a history of multiple strokes and one patient with a history of myocardial infarction who died from cardiac failure. Other serious events include nephrosis and paralysis.

During the Phase 2 Open-Label Study, two patients experienced SAEs, which have not raised any new safety concerns. (Table 4-23)

Table 4-23 Serious Adverse Experiences from Phase 2 Open-Label Study (Japan)

Patient	Age/ Sex	Days of Fabrazyme prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
101	31/M	112	Fever, Limb pain, Malaise, Nasal Congestion	Probable	Infusion-associated reactions
201	16/M	69	Infectious enterogastritis, Severe limb pain	Not Related	Increased rate of hospital-acquired viral infection during this period (pt in hospital since 29 March 2000 for school attendance refusal)
		70	CRP positive (9.5)	Not Related	

4.5.2.3 Serious Adverse Events – Other Studies/Programs

Serious adverse events reported to Genzyme through 01 July 2002 in other ongoing studies with Fabrazyme, compassionate use/special access and spontaneous post-approval. Table 4-24, Table 4-25, Table 4-26, Table 4-27, and Table 4-28 summarize all serious adverse events reported to Genzyme through 01 July 2002 from Phase 1/2 Extension Study, Phase 2 Extension Study (Japan), Phase 4 Placebo-Controlled Study, through Compassionate Use/ Special Access Programs / Other studies, and Post-Approval Spontaneous reports, respectively.

Nine patients have experienced SAEs considered related to treatment with Fabrazyme based on reports received by Genzyme. No new safety concerns have been identified.

Table 4-24 Phase 1/2 Extension Study: Summary of Serious Adverse Events through 01 July 2002

Patient	Age/ Sex	Days of Fabrazyme treatment prior to SAE*	SAE Description	Relationship to Fabrazyme	Medical History
102	47/M	8	Vomiting	Not Related	Hypertension, Anemia, Irritable bowel
	48/M	305	Gallstones	Not Related	
		381	Abdominal Pain, Nausea, Vomiting	Not Related	
103	47/M	76	Atrial fibrillation	Unlikely	Junctional rhythm; Supraventricular tachycardia
104	30/M	92	Uvula Oedema	Not Related	Similar condition in family members
108	40/M	1242	Perforated Diverticulitis, Peritonitis	Not Related	Cramping/diarrhea triggered by fatty foods
110	39/M	32	Chest pain, Fatigue, Dyspnoea	Possible	Cardiac disease & pacemaker

* Patients from the Phase 1/2 Study may not have had continuous treatment between the end of that study and enrollment in the Phase 1/2 Extension Study.

Table 4-25 Phase 2 Extension Study (Japan): Summary of Serious Adverse Events through 1 July 2002

Patient	Age/ Sex	Days of Fabrazyme treatment prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
106	24/M	503	Nausea	Not Related	Holistic treatment for Fabry disease (inflammatory rabbit skin extract) given 4 days prior to SAE
110	32/M	562	Creatinine Increased	Not Related	No relevant medical history

Table 4-26 Phase 4 Placebo-Controlled Study: Serious Adverse Events through 1 July 2002

Patient	Age/ Sex	Treatment Group ^A	Days of study drug treatment prior to SAE	SAE Description	Relationship to Study Medication	Medical History
06043	52/M	Blinded	94	Acute Meniere's attack	Unlikely	Stroke, Angina, CVA (1976).
			337	2 nd Degree Heart Block, Syncope	Unlikely	
14041	65/M	Blinded	54	Hypotension	Definite	Hypertension
18041	51/M	Blinded	28	Cardiac arrest (Section, 4.5.2.1 Deaths)	Unlikely	Hyperlipidemia, gout, kidney stones, findings consistent w/ Fabry disease
20041	36/M	Blinded	40	Chest pain	Unlikely	History of cardiac disease
20046	39/M	Blinded	44	Syncope	Unlikely	Asthma
20047	52/M	Blinded	68	Atrial fibrillation	Unlikely	Pacemaker
24041	54/M	Blinded	105	Chest pain	Unlikely	History of cardiac disease
24810	20/M	Screen failure	0	Acute drug intoxication	Not Related	Narcotic & benzodiazepine use for pain/anxiety.
24811	45/F	Screen failure	0	Acute drug intoxication	Not Related	Prior drug overdose & car accident
25041	52/M	Blinded	43	Angioedema, Flushing, Wheezing, Cough, Urticaria	Possible	Infusion-associated reaction
27041	59/M	Blinded	42	Stroke	Unlikely	History of cardiac disease
30042	33/M	Blinded	81	Mood disorder	Unlikely	Post-traumatic stress disorder and depression
			180	Medication adjustment	Unlikely	
34042	54/M	Blinded	43	Auricular Disease	Unlikely	Cardiovascular: hypertension, left AV bundle-branch block
06047	56/F	Blinded	15	Fever	Not Related	Similar febrile reactions (not during past year)
12041	55/M	Blinded	2	Stroke	Not Related	Ischaemic heart disease, decreased left ventricular function, renal impairment
45041	26/M	Blinded	84	Fabry Pain Crisis	Not Related	Fabry pain & opiate addiction
06042	45/M	Blinded	210	Arthropathy (Swollen knee)	Not Related	Work-related injury
				Arthralgia (Painful knee)	Not Related	
12043	43/M	Blinded	102	Dehydration, Constipation, Vomiting	Not Related	No relevant medical history

^A Treatment assignments have not been unblinded for the Phase 4 Placebo-Controlled Study.

Fabrazyme® (agalsidase beta) BLA STN BL 103979/0

Advisory Committee Briefing Document

Table 4-27 Serious Adverse Events from Special Access, Compassionate Use, and Other Studies (through 1 July 2002)

Source*/ Patient	Age/ Sex	Days of Fabrazyme treatment prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
CU/C101	63/M	185	Ventricular tachycardia (Section, 4.5.2.1 Deaths)	Not Related	History of extensive cardiac disease
CU/C202	46/M	86	Anemia	Unlikely	ESRD, Cerebrovascular, cardiovascular disease
		276	CRP elevated	Not Related	
		299	Ileus	Not Related	
		276	Chest Pain	Not Related	
		295	Swelling (forearms, legs)	Not Related	
		313	Anemia	Not Related	
	47/M	425	Dehydration	Not Related	
ATU/	53/M	42	Ischemic colitis, Multi organ failure (Section, 4.5.2.1 Deaths)	Unlikely	Haemodialysis 3 times a week; abdominal pain
ATU/	36/M	Unknown	Myocardial infarction	Possible	Mild chest pain for 3 yrs
CU/	65/F	170	Retrosternal chest pain	Possible	Cardiomyopathy, stroke, peripheral vascular disease
CU/	44/M	6	Ataxia, Urosepsis	Probable	Cerebellar stroke
CU/	36/M	76	Musculo-skeletal pain	Unlikely	None
CU/	59/M	1	Anasarca, Sepsis (Section, 4.5.2.1 Deaths)	Not Related	Severe Fabry disease; ESRD; Restrictive lung disease; Severe restrictive cardiomyopathy; Chronic atrial fibrillation; Severe ventricular cavity obliteration; pericardial effusion; bilateral pleural effusion; CVA with mild left hemiparesis.
CU/	40/M	19	Rhabdomyolysis	Unlikely	Urine discoloration (red) 26-28 January 2002 thought to be related to rifampicin therapy for infected leg ulcers
MD/	44/M	264	Stroke	Not Related	Three strokes (left hemisphere)
CU/	48/M	51	Chest Pain	Unlikely	Hypertension, Chronic renal failure, Valvular heart disease
CU/	40/M	58	Cellulitis, Gangrene, Pleural Effusion, Bronchitis	Unlikely	Severe Fabry Disease; Depression, Chronic Ulcers; CVA's; Osteoporosis; Rhabdomyolysis (February 2002)
		138	Urinary Tract Infection	Unlikely	
		139	Pulmonary Embolism, Bacterial Infection	Unlikely	

*MD = Physician-sponsored IND (Dr. Linthorst, The Netherlands- Fabrazyme vs. Replagal™ dose = 0.2mg/kg)

CU = Compassionate Use

ATU = France ATU (Autorization Temporaire d'Utilisation)

Table 4-28 Serious Adverse Events - Post-Approval Spontaneous Reports through 1 July 2002

Patient	Age/ Sex	Days of Fabrazyme treatment prior to SAE	SAE Description	Relationship to Fabrazyme	Medical History
—	41/M	121	Infection toe	Unlikely	No related medical history
—	47/M	71	Umbilical hernia	Not Related	History of peritoneal dialysis
—	48/M	20	Cardiac Arrest (Section, 4.5.2.1 Deaths)	Unlikely	History of 4 myocardial infarctions in 1986, 1989, 1997, 1998)
—	14/M	43	Reduced blood pressure, sweating increased, bronchospasm, somnolence	Probable	Infusion-associated reaction
—	36/M	155	Flushing, oedema, pruritus, syncope	Probable	Infusion-associated reaction
—	46/M	306	Cardiac arrest, (Section, 4.5.2.1 Deaths)	Not Related	Congenital heart abnormality; COPD; chronic kidney insufficiency
—	42/M	424	Brain Stem Infarct (Section, 4.5.2.1 Deaths)	Not Related	Kidney Transplant
—	54/M	270	Vascular Encephalopathy	Unlikely	Coronary heart disease; Renal insufficiency with proteinuria; Hypertrophic obstructive cardiomyopathy
—	41/M	322	Facial Oedema, Pruritus, Eye Oedema	Probable	Infusion-associated reaction

^a Fabrazyme dose of all spontaneously reported SAEs was 1.0 mg/kg q14 days

4.5.2.4 Study Discontinuations

No patients in Phase 1/2 Study, Phase 3 Placebo-Controlled Study, or Phase 2 Open-Label Study (Japan) discontinued from study participation due to an adverse event. In the Phase 3 Extension Study (in which patients have received Fabrazyme infusions for 24-30 months), two patients have withdrawn from the study to receive commercial product in the Netherlands and France. Four additional patients have withdrawn as follows (Table 4-29):

Table 4-29 Study Discontinuations

Patient ID	Phase 3 Placebo- Controlled Study Treatment Assignment	Last Infusion Received	Reason for Withdrawal
0104	Fabrazyme	25	Voluntary withdrawal
0304	Placebo	8	Protocol-specified*
0506	Placebo	29	Death
0806	Fabrazyme	36	Protocol-specified*

*Positive skin test

4.5.3 Immunogenicity

4.5.3.1 Seroconversion Rates

It is well known that an immune response may occur following treatment with exogenous human proteins. In the Fabrazyme clinical studies, the majority of patients had low levels of both endogenous plasma α GAL activity and leukocyte α GAL activity at baseline. Possible correlations between endogenous enzyme status and the development of an antibody response (seroconversion) are poorly understood. The development of antibody response is a complex process and needs to be further understood in these patients with genetic deficiencies.

All patients participating in Genzyme-sponsored studies were evaluated for the development of antibodies specific to Fabrazyme (seroconversion).

A very sensitive ELISA was used to quantify the presence of specific antibodies. The assay can detect 0.24 μ g/mL of specific antibody. Given a normal IgG concentration in adult serum of 11.58 mg/mL, this sensitivity limit reflects 0.002% of total IgG in serum. (*Lawlor and Fischer, eds., 1988, Manual of Allergy and Immunology*)

Table 4-30 summarizes the seroconversion rates in the four major studies with Fabrazyme.

Table 4-30 Seroconversion Rates in the Four Major Studies

Study	Number of patients treated with Fabrazyme	Number of patients who seroconverted; (IgG + at anytime)
Phase 1/2 Study	15	8
Phase 3 Placebo-Controlled Study	29	24
Phase 3 Extension Study	58*	52
Phase 2 Open-Label Study (Japan)	13	11

*Includes the 29 patients in the Phase 3 Placebo-Controlled Study

During Phase 1/2 Study, a total 8 of 15 patients seroconverted. Three patients in the 1.0 mg/kg and three patients in the 3.0 mg/kg every 14-day treatment cohorts seroconverted; one of three patients in the both 1.0 mg/kg and 3.0 mg/kg every 48-hour treatment cohorts seroconverted. Immunological response was detected starting at the second or third infusion (14 to 28 days) for patients receiving treatment every 14 days. Seroconversion was detected at the fifth infusion for two patients in the every 48 hour treatment cohorts.

During the Phase 3 Placebo-Controlled Study, there were 24 (83%) patients in the Fabrazyme treatment group and 1 (3%) patient (Patient 116) in the placebo group that IgG seroconverted during the study. Seroconversion was detected at up to Week 20 with the first occurrence of

seroconversion seen at Visit 3. The mean time to seroconversion was observed at the time of the 5th infusion.

During the Phase 3 Extension Study, all patients participating were evaluated for the development of antibodies specific to Fabrazyme. Fifty-two of 58 (90%) patients with up to 24 months exposure had developed IgG antibodies (i.e., any observation of IgG positive results during Phase 3 Placebo-Controlled Study or Phase 3 Extension Study).

During the Phase 2 Open-Label Study, 11 patients (85%) IgG seroconverted. Seroconversion was found at up to Visit 10 with the first seroconversion observed at Visit 3; two patients did not seroconvert. The mean time to seroconversion was observed at the 4th infusion.

4.5.3.2 No Impact of Seroconversion on Efficacy

As of the Phase 3 Extension Study 18-month assessments, 52 of 58 patients (90%) IgG have seroconverted. Of these 52 patients, 44 (85%) seroconverted within 3 months of treatment with Fabrazyme. Despite this high rate of early IgG seroconversion, there was no evidence that the immune response inhibited or neutralized activity of the delivered enzyme from the histologic efficacy parameters (endothelial endpoints in the kidney heart and skin). Specifically, for the 28 patients receiving Fabrazyme in the Phase 3 Placebo-Controlled Study, mean scores for the endothelium of the interstitial capillaries of the kidney, heart and skin showed significant declines in their GL-3 scores at Week 20. (Table 4-31)

Table 4-31 Relationship between IgG Seroconversion and Kidney Efficacy Results at Week 20 of the Phase 3 Placebo Controlled Study

Parameter	Zero-Scores (GL-3 Clearance)	Non-Zero Scores	Total	P-value
Seronegative	4 (80%)	1 (20%)	5	1.000
Seropositive	16 (67%)	8 (33%)	24	

P-value based on Fisher's Exact Test

Additionally, the number of patients achieving zero-scores increased following a further 6 months of treatment in the Phase 3 Extension Study. These findings indicate that the enzyme continued to be effective in the face of persistent IgG titers. Similarly, additional cell types, which included the same three time points, showed the same stepwise reduction in tissue GL-3 between the 6-month and 12-month time points indicating continued efficacy. Furthermore, plasma GL-3 (likely a reflection of total body GL-3 stores) was reduced to undetectable levels, which were generally sustained at all subsequent time points.

4.5.3.3 Immune Complexes

In order to investigate the possible presence of circulating immune complexes (CIC) the QUIDEL CIC-Raji Cell Replacement Enzyme Immunoassay was used.

All 29 patients treated with Fabrazyme during the Phase 3 Placebo-Controlled Study were evaluated for the presence of CIC at baseline, middle and end (Week 20) of study.

Twenty-eight out of 29 patients demonstrated no detectable CIC, with one patient (Patient 115) testing positive for circulating immune complexes as of Week 10. This patient's safety data was reviewed and with the exception of some RBC found in urine (a common finding in Fabry patients), no signs or symptoms were found which suggest the occurrence of immune complex disease.

In addition, kidney biopsy samples from 13 patients obtained at Week 20 of the Phase 3 Placebo-Controlled Study were evaluated and interpreted for the presence of immune complexes by Dr. R. Colvin (Professor of Pathology at Harvard Medical School, and Chairman, Department of Pathology, Massachusetts General Hospital). Only kidney biopsy samples containing glomeruli were included from three groups of patients: a) patients in the Fabrazyme treated group with the highest IgG antibody titers with frozen tissue available (n=5); b) patients in the Fabrazyme treated group with no IgG antibody titers for whom frozen tissue was available (n=3); and c) patients in the placebo group with no IgG antibody titers for whom frozen tissue was available (n=5). All patient specimens were negative for IgG in the glomeruli, and the corresponding complement (C3) levels were recorded as either negative or trace by immunofluorescence, including the samples from five patients treated with placebo. These results suggest that there is no significant immune complex deposition present in the renal glomeruli of these patients. Serologic analyses for circulating immune complexes conducted in the Fabrazyme-treated patients tested negative.

4.5.3.4 Change in Antibody Titers Over Time

Changes in patient antibody titers have been followed over time for patients participating in Phase 3 Extension Study and Phase 2 Extension Study (Japan).

It is important to clarify terminology in the discussion of serology. If a patient did not seroconvert throughout the entire study period, then the patient is defined as having no immune response (seronegative). If a patient seroconverts at any time during the study period and later stops producing IgG antibodies as determined by an ELISA within normal range and two consecutive negative confirmatory RIP assays, then the patient is defined as having tolerized.

If a patient seroconverted at anytime during the entire study period and the highest titer value is less than or equal to 800, then the patient is defined as a low titer immune responder ("low responders"). Excluding the low responders, a \geq four-fold decrease in titer from the peak measurement to the last

measurement is considered a downward trend. Patients whose highest titer to date was achieved at the last visit are included in a separate category. Patients who were not defined in any of the above categories are defined as patients whose titers have plateaued. (Table 4-32)

Table 4-32 Summary of IgG Titer Categorization for Phase 2 Extension Study (Japan) (through February 2002) and Phase 3 Extension Study (as of the 24 Month Timepoint)

	Total patients*	Seronegative	Low responder	Downward Trend	Plateau	Highest titer to date	Tolerized
Phase 3 Extension (24 months)	57	6	2	25	15	2	7
Phase 2 Extension (Japan)	13	1	4	4	4	0	0

* Number reflects total number of patient titers available at analysis/categorization.

Phase 3 Extension Study

One patient (Patient 304) was not included in this longitudinal analysis because he withdrew from the Phase 3 Extension Study after 14 weeks of participation and comparative antibody titers are only available for a short duration of time. The serology status of the remaining 57 patients who have received treatment with Fabrazyme for 24-30 months indicates that approximately 10% of patients continue to be IgG seronegative.

Two (2) of the 51 patients who seroconverted (IgG positive) are considered low responders (IgG titers not exceeding 800). Two patients' highest titer to date was at the last visit. A review of these patients' adverse event profiles continues to primarily consist of mild febrile reactions (e.g., fever, chills) in addition to reports of mild temperature change sensation and shortness of breath. Despite the increase in antibody titers, there has been no demonstrable decline in efficacy. These patients' renal function as evidenced by serum creatinine has remained stable. Among the remaining IgG positive patients, 25/40 (63%) have demonstrated a downward trend in antibody titer based on a ≥ four-fold reduction in titer from the peak measurement to the last measurement. The remaining 15/40 (38%) patients demonstrated a plateau in their antibody titers.

As of the 24-month timepoint, seven of the 52 seroconverted patients (13.5%) have two negative confirmatory RIP and are considered to have "tolerized" (stopped producing IgG antibody).

Phase 2 Open-Label Study (Japan)

The serology status of the 13 Japanese patients who participated in the Phase 2 Open-Label Study (Japan) (including data available in the extension study) indicates one patient continues to be seronegative.

Four of the remaining 12 patients who seroconverted are considered low responders (IgG titers not exceeding 800; one patient seroconverted during the extension study). Four patients have demonstrated a downward trend in antibody titer based on a \geq four-fold reduction in titer from the peak measurement to the last measurement. The remaining four patients demonstrate a plateau in their antibody titers.

4.5.3.5 IgE and Experience with Fabrazyme Rechallenge

The number of patients who have actually tested positive for IgE using an enzyme-linked immunosorbent assay is very small. To date, two patients have been withdrawn from Genzyme-sponsored clinical studies (per protocol requirements) due to the detection of IgE antibodies in serum and three patients have been withdrawn as a result of positive skin testing. There are no reports of anaphylaxis.

Classical Fabry is a progressive disease that often culminates in renal failure, cardiac failure and/or stroke resulting in increased morbidity and mortality. The benefits of a potentially disease-altering therapy may be thought to outweigh the possible risks in these IgE positive and skin test positive patients when these risks are minimized by careful administration of Fabrazyme in this sensitized population.

This concept is being studied using a Genzyme-sponsored cautious rechallenge protocol. The general strategy is to administer low doses of Fabrazyme with incremental progression at regular intervals until a full therapeutic dose is achieved. To date, two (2) skin test positive and one (1) serum IgE positive patient have been rechallenged under this protocol and one is pending treatment.

- One positive skin test patient and one serum IgE positive patient have successfully repeatedly received Fabrazyme without incident following the dose and infusion rate schedule outlined in the protocol.
- One positive skin test patient experienced symptoms during the first rechallenge infusion consistent with previous reactions and the infusion was stopped. The patient was not considered to have experienced anaphylaxis. Blood samples collected prior to, at multiple time points during and twice after the infusion all tested within normal range for serum tryptase providing evidence that the patient's symptoms were not mediated by IgE antibodies. The patient is scheduled to resume the cautious rechallenge shortly.

In addition, one study patient whose sera tested IgE positive during Phase 3 Extension Study (and was subsequently withdrawn per protocol), has successfully been rechallenged repeatedly using

commercially available Fabrazyme, with a oral pre-treatment regimen consisting of prednisone, hydroxyzine and paracetamol.

4.5.4 Laboratory And Other Test Abnormalities

Laboratory parameters including ophthalmologic, ECG and echocardiogram findings were measured and analyzed for each study. While various specific tests were outside of the normal ranges in some studies and were captured as adverse events, no sustained and/or clinically relevant changes were observed for any of these parameters over time. No pattern has been demonstrated that indicates treatment with Fabrazyme has a toxic effect.

4.5.5 Summary of Safety

- Full safety data derived from the treatment of 71 patients in three major studies (Phase 3 Placebo-Controlled Study, Phase 3 Extension Study, and Phase 2 Open-Label Study (Japan)) demonstrate that patients tolerate the long-term use of Fabrazyme.
- The majority of patients develop IgG antibodies during treatment. Among IgG positive patients in Phase 3 Placebo-Controlled Study whose antibody data have been followed for approximately 24-30 months in Phase 3 Extension Study, over half have experienced a four-fold reduction in titer with continued treatment. Similar findings have been observed in patients enrolled in the Phase 2 Open-Label Study (Japan). Immune tolerance has been achieved in seven patients.
- The proportion of patients who IgG seroconverted is almost identical in the Fabrazyme patients in the Phase 3 Placebo-Controlled Study (83%) and Phase 2 Open-Label Study (Japan) (85%). IgG seroconversion does not impact efficacy as assessed by i) sustained clearance of tissue GL-3 as measured by light microscopy; ii) sustained clearance of plasma GL-3; and iii) stable renal function.
- There are no reports of anaphylaxis. Two patients tested serum IgE positive and three patients tested skin test positive representing < 1.4% (5 of >350) of all treated patients. One patient has been successfully rechallenged repeatedly with commercial product, three patients have been treated under Genzyme's cautious graded rechallenge protocol and the fifth patient is pending rechallenge.
- Long-term treatment has demonstrated a progressive decrease in the number of patients with infusion-associated reactions. In addition, long-term data have demonstrated patients are able to successfully tolerate increases in infusion rate resulting in shortening in the total infusion time.
- Results from laboratory tests are similar across all studies and indicate that treatment with Fabrazyme appears to have no toxic effect. Chronic therapy is generally well tolerated.

4.5.6 Safety Conclusion

It is estimated that over 350 patients have received over 4000 total infusions of Fabrazyme (including patient experience with Fabrazyme through compassionate use/special access, other on-going studies and post-approval) with the longest patient on therapy for over 3 years. Continued treatment with Fabrazyme has not been precluded by IgG antibody development and no demonstrable effect on efficacy has been observed.

Long-term safety data representing 18-24 months of treatment with Fabrazyme in addition to Fabrazyme experience through other on-going studies, compassionate use/special access and spontaneous post-approval continue to demonstrate Fabrazyme infusions are generally well-tolerated.

5. RISK/BENEFIT

5.1 Fabry Disease and Current Medical Care

In classically affected individuals, the phenotypic expression of Fabry disease is manifested as decreased or absent activity of α -galactosidase A, resulting in the pathological accumulation of α -galactosyl-terminated neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3) in cellular lysosomes. Accumulation occurs in virtually all tissues of the body, but particularly in the endothelial cells leading to end-organ damage of the kidney, heart, and brain.

Currently no specific treatment or cure exists for Fabry disease; therefore, therapy is aimed at sign and symptom palliation. However, the course of disease progression (renal, cardiac and cerebrovascular) is largely unaffected by current medical interventions. Pain management continues to be a large part of the medical therapy of Fabry disease. Opiates, diphenylhydantoin, carbamazepine and gabapentin have all been used with varying degrees of success (*Lockman, 1973, Neurology*) (*Lenoir, 1977, Arch Franc Ped*) (*Filling-Katz, 1989, Neurology*) (*Inagaki, 1992, Brain Dev*) (*Desnick, 1995, in The Metabolic Basis of Inherited Disease*) (*Peters, 1997, Postgrad Med*). Oral anticoagulants and/or antiplatelet therapy are recommended for stroke-prone patients. Dialysis and/or renal transplantation are the two currently available therapeutic options for end-stage renal disease.

5.2 Fabrazyme Benefits

Fabrazyme contains the active ingredient recombinant human α -galactosidase (agalsidase beta). It was developed to restore the endogenous enzyme activity lacking in patients with Fabry disease (enzyme replacement therapy).

Enzyme replacement therapy with Fabrazyme has been demonstrated to clear pathologic GL-3 accumulations to normal or near-normal levels in multiple cell-types. Specifically GL-3 has been cleared to normal or near-normal levels in the critical cell types of the kidney that are involved in the renal pathophysiology of the disease. We have hypothesized that prolonged treatment with Fabrazyme resulting in removal of GL-3 accumulation will lead to stabilization or possible improvement in organ function.

Currently, 148 patients are participating in ongoing clinical studies that will provide additional long-term clinical outcome data to support the histologic findings and confirm the hypothesis that treatment with Fabrazyme leads to stabilization or improvement in renal function and other clinical manifestations of the disease.

5.3 Risks Associated with Fabrazyme Therapy

In the setting of an endogenous enzyme deficiency, infusion of an exogenous recombinant replacement enzyme may be expected to cause immune reactivity to the enzyme.

Full safety data derived from the treatment of 71 patients in three studies demonstrate that patients are able to tolerate the long-term use of Fabrazyme. Although the majority of patients develop antibodies to Fabrazyme, this does not preclude continued treatment.

Approximately 90% of patients receiving Fabrazyme 1.0 mg/kg every 2 weeks IgG seroconverted; however, continued treatment with Fabrazyme has not been precluded by IgG antibody development and there is no observable impact on efficacy. Among IgG positive patients in Phase 3 Placebo-Controlled Study whose antibody data have been followed through 24 months in the Phase 3 Extension Study, almost half have experienced a four-fold reduction in titer with continued treatment. Immune tolerance has been achieved in seven patients. The proportion of patients who IgG seroconverted is almost identical in the Fabrazyme patients in the Phase 3 Placebo-Controlled Study (83%) and Phase 2 Open-Label Study (Japan) (85%).

There have been no reports of anaphylaxis. Two patients tested serum IgE positive, and three patients tested skin test positive representing < 1.4% of all treated patients. One patient has been repeatedly rechallenged successfully with commercial product, three have been treated in Genzyme's cautious graded rechallenge protocol, and one is pending rechallenge.

Results from laboratory tests are similar across all studies and indicate that treatment with Fabrazyme appears to have no toxic effect. Ophthalmic, ECG, and echocardiogram findings further support this observation.

Long-term treatment has demonstrated a progressive decrease in the number of patients with infusion-associated reactions. In addition, long-term data have demonstrated patients are able to successfully tolerate increases in infusion rate resulting in shortening in the total infusion time.

5.4 Conclusions

Currently, no treatment is able to prevent or impede the progressive vascular damage and resultant end organ destruction of Fabry disease. Consideration must be given to the serious and often lethal manifestations of Fabry disease, the minor risks associated with Fabrazyme treatment, the effect on clearance of GL-3 to normal or near-normal levels in critical cells involved in the pathophysiology of the disease, and the potential for long-term clinical benefit. The data demonstrate robust efficacy and an acceptable safety profile for Fabrazyme therapy; therefore, accelerated approval is justified.

The Phase 4 study has been a focus of the FDA questions for Fabrazyme, relating to such issues as the appropriate control and the feasibility of conducting a long-term study in a post-marketing setting. In the following sections, we describe the ongoing Phase 4 study as well as important modifications to the Phase 4 Program that we have proposed to FDA and with which we would like to proceed.

6. PHASE 4 POST-APPROVAL CLINICAL PROGRAM TO VERIFY CLINICAL BENEFIT

Genzyme has requested an accelerated approval pursuant to 21 CFR Part 601, Subpart E:

These regulations provide a mechanism by which products that offer meaningful therapeutic benefits over existing treatments for serious, life-threatening illnesses are made available (marketing approval granted) as soon as possible based on demonstrated effects on surrogate endpoints (reasonable likely to predict clinical benefit) while confirmatory clinical studies are conducted to verify and describe the clinical benefits.

Approval under this regulation is subject to the requirement that the applicant study the biological product further to verify and describe the clinical benefit where there is uncertainty as to the relation of the surrogate endpoint to the clinical benefit. These confirmatory clinical studies must be adequate and well-controlled and would usually be underway at the time of approval of the BLA. For biological products approved under these regulations FDA may withdraw approval if the sponsor fails to conduct the post-marketing clinical study(ies) or if the study(ies) fails to verify clinical benefit.

Genzyme has conducted, completed and reported the results of the Phase 3 Placebo-controlled, adequate and well-controlled clinical study and established that Fabrazyme has an effect on a surrogate endpoint that is reasonable likely, on the basis of pathophysiologic evidence, to predict a clinical benefit. It could be argued that the submitted evidence goes beyond a surrogate endpoint and is tantamount to clinical evidence. The histologic evidence presented in the BLA is unequivocal and evidence of a dramatic effect on the underlying pathology of the disease. There are other examples of the value of histology in objectively assessing disease progression and response—myocardial biopsies in myocarditis and renal biopsies in renal transplantation to assess organ rejection.

In addition, Genzyme has designed, submitted and fully enrolled an adequate, well-controlled clinical trial (multicenter, randomized, double-blind, placebo-controlled) to verify and describe the clinical benefit. This placebo controlled study design was proposed in August 2000 because no alternatives existed at that time for meeting FDA's requirement that the study would be well-controlled. In the following sections Genzyme describes this trial (referred to as Phase 4 Clinical Study), the concerns raised by CBER regarding this trial, and alternative proposals that address these concerns.

Genzyme wants to stress that these FDA concerns that are currently preventing the approval of Fabrazyme are addressable in various ways and, more importantly, are the types of issues that can and should be addressed in a post approval setting as intended by the cited regulations. Genzyme asks the Committee to recommend approval of the Fabrazyme application on the basis of the

submitted evidence and direct the FDA and sponsor to resolve the issues regarding the method of control and the analysis of the Phase 4 trial in a post-approval setting while granting access to this important therapy for the very small but seriously ill patient population with no other alternative treatment options.

6.1 Current Phase 4 Study (Genzyme Study AGAL-008-00)

The key design elements of the ongoing Phase 4 Study are as follows:

- **Study Design:** Multi-center, randomized, double-blind, placebo-controlled study
- **Primary Endpoint:** Time to progression of renal disease, cardiac disease, cerebrovascular disease, and/or death. If a patient in either arm experiences a clinically significant event that the Principal Investigator feels meets the criteria of a primary endpoint, a blinded Independent Adjudication Board will review the event.
- **Number of Patients and Study Centers:** The protocol design was based on an enrollment of 70 patients (76 patients have been randomized) to one of two treatments at approximately 30 study centers.
- **Treatment Regimen and Treatment Assignment:** Patients will receive 1.0 mg/kg of Fabrazyme or placebo every 2 weeks, based on a 2:1 (Fabrazyme:placebo) randomization scheme. Patients assigned to either treatment group will continue to receive standard care and are monitored closely for progression of disease.
- **Study Duration:** Approximately 35 months (based on the second interim analysis) or until the required number of clinical events (renal events) is achieved to reach statistical significance. The clinical portion of the study will be concluded in early 2004 based on the current estimate.
- **Critical Inclusion Criteria and Rationale:** Baseline serum creatinine in the range of 1.2 to 3.0 mg/dL or creatinine clearance < 80 mL/min if serum creatinine < 1.2 mg/dL. Based upon review of the limited available historical data, it was clear that in order to demonstrate within a reasonable timeframe the potential clinical benefit of Fabrazyme with respect to preservation of renal function versus an untreated control group, it was necessary to study patients who had already begun to manifest renal insufficiency.

This study is event driven where an event is defined by the first occurrence of any one of the predefined clinically significant renal, cardiac, cerebral vascular events and/or death. For example, the definition of a renal event is a 33% increase in serum creatinine values over baseline or progression to dialysis or renal transplantation. A successful study outcome is a statistically significant decrease in the event rate for patients on Fabrazyme compared to placebo.

Because of the limited historical data available at the time the study was initiated in the first Quarter of 2001, the study includes blinded interim analyses to allow for the adjustment of the sample size and/or study duration depending on the observed event rate. One additional point deserves mention. The original goal of this study was to verify the clinical benefit of Fabrazyme with respect to decreasing the rate of progression of Fabry renal disease. However, because investigators were concerned about the potential of maintaining patients on placebo in the face of cardiac and/or cerebrovascular disease progression (though renal disease might not have progressed significantly), the primary endpoint was modified. To accommodate this concern of the investigators, the composite endpoint described above was developed. The composite endpoint is considered somewhat problematic in that non-renal events (cardiac and stroke) occurring early in the study, are counted toward the event rate, though it is certainly unreasonable to expect that Fabrazyme would have a positive treatment effect in such a short timeframe.

6.1.1 Status of Current Study

Extensive resources have been devoted to this Phase 4 study and much progress has been made. The study is currently fully enrolled, with the first patient having received treatment for approximately 21 months. Over 235 patients have been consented and screened at 34 different sites worldwide. Seventy-six patients have met enrollment criteria and have been randomized and infused. (See Table 6-1)

Table 6-1 Summary of Phase 4 Study Progress in Site and Patient Enrollment

Study Sites Approached to Participate	60
Study Sites that Chose Not to Participate	26 ^A
Study Sites that are Screening Patients	34 (US, Canada, Australia, Czech Republic, Hungary, Poland, Uruguay, United Kingdom,
Patients Consented and Screened for Enrollment	235 ^B
Patients who Failed Screening	154 (primarily due to out-of-range serum creatinine levels)
Patients Currently Enrolled	76
A Reasons for not participating included: a placebo-controlled study was perceived to be unethical; the site did not have any patients; and positive opinion and approval to market was granted in Europe.	
B Some patients have not completed their Screening assessments.	

6.1.2 Current Study Design Issues

In several discussions between Genzyme, FDA, investigators, and other experts, concerns have been raised about several aspects of the current, placebo-controlled, Phase 4 study. These concerns primarily involve the ethics and feasibility of completing a long-term placebo-

controlled study in a post-marketing setting with an endpoint of irreversible organ damage; and that the post-marketing study might yield inconclusive results creating uncertainty as to the clinical benefit and possible withdrawal of the product that might, in fact be beneficial.

With respect to patient retention in the placebo-controlled Phase 4 study once marketing approval is granted, Genzyme has received explicit commitments from the site investigators regarding their intention to complete the study post-approval. Genzyme has also changed the Informed Consent Form so that patients are aware and acknowledge the expected study length of up to 35 months or until a significant medical event. However, there is no way of guaranteeing that patient retention will not be a problem in the post-approval setting. In addition, 21 CFR 50.25(a)(8) requires that patients be informed that they can withdraw from the study at any time. This is further magnified by the fact that end organ damage that does occur while a patient may be on placebo is likely to be irreversible, thus possibly raising ethical concerns.

As an alternative to the placebo-controlled study, Genzyme has gone to great lengths to collect and organize a substantial natural history database (See Section 6.3) and proposed to CBER the conversion of the study to a single-arm historically-controlled study (see Section 6.2). In addition the new Phase 4 program allows Genzyme to study a much broader cross population of Fabry patients than the current study which, in order to complete within a reasonable time frame of 2-3 years, currently only studies a very small subset of Fabry patients with mild to moderate renal insufficiency.

6.2 Proposal for New Phase 4 Clinical Program

Because of the issues noted in Section 6.1.2 above and taking into account the inevitable problems related to the demonstration of a long term benefit in this rare and slowly progressive disease, Genzyme is proposing a modified and expanded Phase 4 program. This modified Phase 4 program would include extensive study of the impact of Fabrazyme on a broader cross-section of the Fabry population than is currently being studied in the placebo-controlled Phase 4 study (e.g. patients with serum creatinine levels of 1.2 to 3.0). This new four-pronged program, which is described below addresses each of the concerns listed above and therefore accelerated approval based on this proposed Phase 4 program, which is currently fully enrolled and underway, can be granted.

1. Develop a prospectively defined, comprehensive natural history database.

- The collection of these data under a prospectively defined protocol is complete. The final study report was submitted to the BLA on October 18, 2002. The natural history database consists of 447 unique patients from 27 sites in 5 countries. The data from

the Historical Study are recent, with 75% of the serum creatinine measurements in the “qualified patients” occurring after April 1996. The contemporaneous nature of the historical data suggests that statistical models can be utilized that would provide a robust assessment of the data. (See Section 6.4.1 for further details and results).

2. Utilize an appropriate subset of patients from the natural history database as a control group to convert the current Phase 4 Placebo-Controlled study into a single-arm, historical-controlled study by comparing the renal disease event rates in this relatively advanced disease population.

- The proposed protocol (Genzyme Study AGAL-008-00) for the conversion of the study was submitted to FDA in April 2002. The protocol synopsis is included in Appendix 8.1.
- Whereas the placebo-controlled study focused on a composite endpoint consisting of renal, cardiac, cerebrovascular, and death components, the focus of the active treatment protocol will be improvement in renal outcomes.
- Converting the placebo-control design to a single-arm, active treatment design obviates any concerns about the feasibility or ethics of maintaining Fabry patients on placebo in a post-marketing setting and allows the study to focus on progression of renal disease, the most common devastating manifestation of Fabry disease.
- Appropriate statistical methodology can be utilized to analyze the outcomes in the Fabrazyme treated and historical control Fabry patients. (See Sections 6.4.1 and 6.4.2 for further details.)

3. Utilize an appropriate subset of patients from the natural history database to compare the event rate of renal disease in the 58 Fabry patients enrolled in the Phase 3 Extension study (Genzyme Study AGAL-005-99).

- The patients in the Phase 3 Extension study represent patients with much less advanced renal disease.
- The patients in the the Phase 3 Extension Study will be followed for at least 5 years from the date of enrollment in the Phase 3 Placebo-Controlled Study.
- An interim analysis of the Phase 3 Extension Study patients after 24-30 months on Fabrazyme therapy was completed in November, 2002. The interim analysis shows encouraging trends with respect to slowing the progression of renal functional decline in patients receiving Fabrazyme for up to 30 months. (See Section 4.2.1.5)

4. Commit to an extensive Fabry Registry program (already in place worldwide)

- The Fabry Registry will be open to any Fabry patient regardless of treatment and is intended to collect long-term data with the express purpose of expanding the knowledge of Fabry disease and treatment with Fabrazyme.

This multi-faceted approach provides additional confidence and obviates concerns regarding inconclusive results from any one element.

6.2.1 Rationale for Historical Control Phase 4 Study

The purpose of conducting clinical investigations of a drug is to distinguish the effects of the drug from other influences such as spontaneous change in the course of the disease, placebo effect or biased observation. An adequate and well-controlled clinical study has several characteristics, one of which is that the design used must permit a valid comparison with a control group in order to provide an adequate assessment of drug effect. FDA and ICH guidelines recognize historical controlled studies in the hierarchy of clinical study designs that are considered adequate and well-controlled. There have been cautions raised about the use of historical controlled efficacy studies with regard to such points as whether disease progression can be characterized sufficiently, whether there are differences in diagnoses, follow-up, frequencies of measurements, concomitant medications or treatment paradigms. However, historical controlled studies have been used successfully in settings in which the disease is rare, there is a high likelihood of a particular outcome in the absence of therapy and the new intervention can produce dramatic results.

Furthermore, in the matter at hand, Genzyme is not proposing the single-arm, historical-controlled trial in a vacuum to demonstrate safety and effectiveness of Fabrazyme for the prevention of progression of renal impairment in Fabry patients. Genzyme has conducted and reported the unequivocal results of the pivotal Phase 3 placebo-controlled clinical trial demonstrating the safety and effectiveness of Fabrazyme as determined on a histologically assessed and related renal vascular endpoint. The proposed single-arm, historical-controlled trial is a confirmatory study to verify and describe the clinical benefit that will likely be derived on the basis of the effect of enzyme replacement on the histopathology of the disease. When one considers the extremely rare nature of the disease, the existing clinical and preclinical data attesting to the effect of enzyme replacement on the underlying histopathology and the inherent complexities of conducting long-term (35 months) placebo-controlled trials in this severely ill patient population, one must approach the issue with flexibility and reason.

- ICH Guidelines (E-10, Choice of Control Group and Related Issues in Clinical Trials) also describe the circumstances under which Historical Control Trials can be used in demonstrating the efficacy of a treatment:

“Externally (Historical) controlled trials are most likely to be persuasive when the study endpoint is objective, when the outcome on treatment is markedly different from that of the historical control and a high level of statistical significance for the treatment-control comparison is attained, when the covariates influencing outcome of the disease are well characterized, and when the control closely resembles the study group in all known relevant baseline, treatment (other than study drug), and observational variables.”

The proposed active treatment historical control study meets the requirements as described in the ICH Guidelines (E-10, Choice of Control Group and Related Issues in Clinical Trials). For example, the primary endpoint is an objective parameter (renal event rates); two groups would be matched based on inclusion/exclusion criteria; the medical history background for the historical control group is reasonably well defined and the treatment effect size is reasonably large (Table 6-3).

Genzyme believes that the historical-control design can establish the clinical benefit of treatment with Fabrazyme and believes that the current data from the Natural History Study supports the proposal to convert the Phase 4 study to an active treatment, historical control design.

6.3 Epidemiological Study of the Natural History of Fabry Disease

In order to develop an extensive, unbiased assessment of the natural history of Fabry disease, Genzyme Corporation sponsored a prospectively defined, epidemiological study of the natural history of Fabry disease (Genzyme Study AGAL-014-01). A final study report was submitted to FDA in October 2002 (Appendix 8.3). An outline of the study methodology and data collected is provided below. In Section 6.4, two alternative statistical methods of analysis are discussed for use in comparing the event rate for patients in this natural history database with Fabrazyme treated patients from the Phase 3 Extension and Phase 4 clinical studies.

6.3.1 Study Methodology

This was an international, multicenter study of an historical cohort of patients with Fabry disease. The objective of the study was to develop a data base from Fabry patient medical record data which could be used to (i) allow more accurate determinations of event rate and sample size estimates for the Phase 4 study, and (ii) to create historical control groups of patients with Fabry disease who fit the inclusion and exclusion criteria of ongoing and future Genzyme studies with Fabrazyme. Medical records were reviewed from as many patients with Fabry disease as possible.

Three key steps have been taken in order to minimize bias in the collection of data from patients' medical records.

- First, data were abstracted under the supervision of an independent contract research organization (Abt Associates Clinical Trials (AACT), 55 Wheeler Street, Cambridge, MA 02138). This organization was identified as an expert in methodologies of collecting epidemiologic and survey data.
- Second, 51 domestic and international investigator sites had been given an opportunity to participate in the study; 27 elected to participate and provided data.
- Third, clinically relevant information on Fabry patients was collected with prospectively defined data points.

Additionally, the quality of data was ensured by quality control measures on the part of the clinical research associates (CRAs) who abstracted the patients' records. The first two records from each CRA were re-abstracted by an experienced supervisor. Any discrepancies noted by the supervisor were made apparent to the CRA, and were recorded and initialed on the original abstraction. Subsequently, approximately 10% of each CRA's abstractions were randomly chosen and re-abstracted by supervisors. Further, if patient data were available at more than one site, the data were collected, duplicates were identified, and the patients' records were merged.

6.3.2 Data Collected

Medical records were abstracted for a total of 447 unique Fabry patients from a total of 27 participating sites in 5 countries. Of the 447 unique patients in the historical database, relatively large subsets were identified that could be used as historical control populations to compare renal event rates for patients treated with Fabrazyme as part of the Phase 3 Extension Study and the Phase 4 study. The precise size of the historical control groups depends upon the statistical methods utilized. For example, 104 patients have data available that met the inclusion/exclusion criteria of the Phase 4 Study and 190 patients had data available that met the inclusion/exclusion criteria of the Phase 3 Placebo-Controlled/Extension Studies. In addition the data collected was contemporaneous in nature, for example of the 104 Phase 4 "qualified patients", 75% of the serum creatinine measurements occurred after April 1996.

6.4 Creation of Fabry Patient Historical Control Groups (from Natural History Database) for Comparison to Patients in the Phase 3 and Phase 4 studies

Distinct statistical methodologies exist which may be employed to (1) derive appropriate historical control groups from the 447 Fabry Natural History Database, and (2) compare the historical control group to the active treatment group with respect to renal event rates. The first

method presented (Section 1.4.1) is the Linear Random Effects Model that was initially submitted to FDA in June 2002 (final report October 2002; Appendix 8.3). FDA raised a number of questions and concerns regarding the application of this methodology to the natural history data. During discussions in September 2002, (after the cancellation of the Advisory Committee planned for September 26th) Genzyme was encouraged by the FDA to pursue the assessment and discussion of alternative methods of analysis with the hope that an agreement could be reached between Genzyme and FDA, on the plan for moving forward. Therefore, Genzyme, in collaboration with Dr. Donald B. Rubin, John L. Loeb Professor of Statistics, Chairman Department of Statistics, Harvard University, has continued to explore alternative approaches for the statistical analysis of the natural history data with the Phase 4 and Phase 3 Extension study patients. As a result, a second statistical method is presented (Section 6.4.2) which employs matching the historical control study group to the clinical study patient group using propensity scoring algorithms.

6.4.1 Linear Random Effects Models

In the Fabry Disease Natural History final study report submitted to FDA in October 2002, renal event rates are estimated using linear random effects models based on the underlying patient-specific trend in serum creatinine over the full duration of follow-up. The empirical estimate based on tabulating the proportion of patients who were observed to have had a 33% increase in serum creatinine, or who required dialysis (length > 40 days), or kidney transplantation within the first 2 years from the Qualification Start Date was biased as some patients had less than 2 years follow-up. This was because patients who had been followed for fewer than 2 years had a lower probability of an event, and inclusion of these patients in the empirical estimation would underestimate the event rate. In order to have more precise and robust estimates of the event rate, a linear random effects model was employed.

The model demonstrated that log-serum creatinine depended on the value at entry (intercept) and time since entry. The linear rate of change in log-serum creatinine over time (slope) depended on the patient. The model was fit by the method of restricted maximum likelihood, and the underlying patient-specific trend is determined from the empirical Bayes estimates of the random intercept and slope. On the log scale, the expected percent change in serum creatinine is directly related to the individual patient slope.

A linear random effects model was fit to the data of the AGAL-008-00 (Phase 4) Qualifiers, using the method of restricted maximum likelihood estimation. Individual patient slopes were estimated using empirical Bayes estimation (*Laird and Ware 1982, Biometrics*). The 80%, 84%, 90%, and 95% confidence intervals on the event rate were estimated.

The primary model to determine the estimated renal event rate was a linear trend random effects model for log serum creatinine. Two criteria for determining a renal event were examined, a patient having an estimated 33% increase from baseline in serum creatinine within 2-years, and a patient having an estimated 50% increase within 3-years.

The key results from the analyses on the 104 patients who met the entry criteria of the Phase 4 Study (“Qualified Patients”) were that 32% of patients with a serum creatinine level of 1.2 – 3.0 mg/dL have an estimated 50% increase in serum creatinine from baseline within 3 years. In addition, 30% of these patients have an estimated 33% increase in serum creatinine from baseline within 2-years. (Table 6-2)

Table 6-2 Estimated Event Rate for Renal Events Based on n = 104 and the Random Effects Model

Serum Creatinine Increase	Estimated Event Rate
33% Increase Over 2 Years	30%
50% Increase Over 3 Years	32%

It is proposed that the estimated 3-year renal event rate from the natural history study and corresponding confidence interval be used as a comparison to the Phase 4 study. The renal event rate of the single arm Phase 4 Fabrazyme® study will be determined to be different from the natural history study if their corresponding 84% confidence intervals are non-overlapping. It should be noted that non-overlapping 84% Confidence Intervals for the individual renal event rate proportions are approximately equivalent to the corresponding 95% Confidence Interval for the difference of the parameters not including zero.

For the Phase 4 study, Fabrazyme treated patients, exact 80%, 84%, 90%, and 95% confidence intervals were calculated for various projected event rates (Table 6-3). The calculations were based on a historical control population of n=104 and on the projected enrollment of n=70 for the Phase 4 study.

Table 6-3: A Comparison of the Estimated Three-Year Event Rates from Historical Control Population versus the Projected Three-Year Event Rates from r-hαGAL (AGAL-008-00) Treated Patients

Confidence Interval	Estimated 3-yr Event Rate (50% increase in serum creatinine) in Historical Database AGAL-008-00 Qualifiers (N=104)	Projected 3-yr Event Rate (50% increase in serum creatinine) in Phase 4 Study Patients (N=final projected enrollment of 70)*		
		10%	15%	20%
	32%	10%	15%	20%
80%	25, 36	6, 16	10, 23	14, 27
84%	25, 36	5, 17	10, 23	13, 28
90%	24, 36	5, 18	9, 25	13, 29
95%	24, 40	4, 20	8, 26	11, 31

The confidence interval for the historical control event rate is based on the confidence interval for the slope parameter in the linear random effects model. The confidence interval for the projected event rate is based on a two-tailed Fisher-Exact test.
* 76 actually enrolled

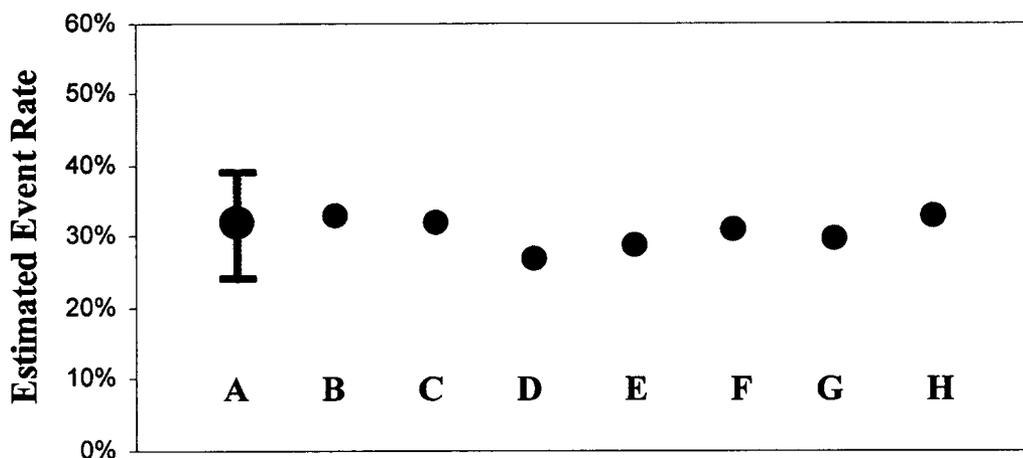
The following supplementary analyses were also performed to assess the robustness of the renal event rate:

- Re-analyzing the data by omitting patients with limited observations (patients with ≥ 2 observations, patients with ≥ 3 observations)
- Adjusting for covariates (age, gender, weight, blood type, and plasma α -GAL level)
- Empirical estimates of renal event rate
- Inclusion of a quadratic term in the statistical models
- Investigation of 1/serum creatinine transformation
- By-subject linear regression
- Analysis of estimated GFR
- Justification of the natural logarithm transformation for serum creatinine.
- Justification of 84% confidence intervals for the event rates.
- A likelihood based approach to the renal event estimate.
- Exclusionary subset analyses.

- Impact of ACE Inhibitors/Angiotensin Receptor Blocking agents on change in serum creatinine over time.

The modeling scenarios conducted by Genzyme, including those conducted to address FDA questions, are remarkably consistent when comparing the Qualified Population (patients who have qualified for Genzyme Phase 4 Study) and the subsets of that population (see Figure 6-1 and Appendix 8.3). Therefore, Genzyme proposes that this database could serve as a valid comparator to the Phase 4 study where a comparison could be made between the historical renal event rate and the Phase 4 renal event rate to demonstrate the efficacy of Fabrazyme. The results of the analyses are included in the Final Report of the Epidemiologic Study of the Natural History of Fabry Disease dated October 10, 2002, submitted to the BLA on October 18, 2002.

Figure 6-1 Comparison of Estimated 3-Year Renal Event Rate Demonstrating Consistency Using Different Statistical Modeling Scenarios



Supplementary Analysis Groups

- | | | |
|------------------------------------|-------------------|---------------|
| A: Linear Random Effects (Primary) | D: ≥ 2 Obs | G: Regression |
| B: Quad. Term | E: Empirical | H: Likelihood |
| C: 1/Serum Creatinine | F: Cov: Blood Grp | |

6.4.2 Assessing the Efficacy of Fabrazyme in a Single Arm Phase 4 study by using a Matching historical Control Study Group using Propensity Scoring Algorithms

Genzyme, in collaboration with Dr. Donald B. Rubin, John L. Loeb Professor of Statistics, Chairman Department of Statistics, Harvard University, has explored alternative approaches for assessing the clinical benefit of Fabrazyme employing the natural history data base as a comparator to the Fabrazyme treated patients in the Phase 4 study using a “matching historical

control study group”. An important value of this approach is that it addresses the issue raised by FDA concerning representativeness and missing/nonuniformly distributed serum creatinine values of the historical database for use as a control for the Phase 4 study. The full statistical analysis plan was submitted to the BLA on November 20, 2002 (see Appendix 8.4). This approach is described in the following section as it applies to the patients with relatively advanced renal disease in the Phase 4 study. An analogous approach is also planned to be applied to the patients with less advanced renal disease currently being treated with Fabrazyme in the Phase 3 Extension Study.

The objective of this analysis will be to compare the renal event rate in a Fabrazyme® treated population to a matched control group. For this analysis a renal event involves the assessment of the increase from baseline in serum creatinine within three years. Specifically, the objective will be to compare the 76 patients in the phase 4 randomized double-blind AGAL-008-00 study to an appropriately matched control group from the AGAL-014-01 historical control study. Two comparisons are implied by this plan. First, the comparison of the renal event rate of the randomized treated patients to the matched historical controls. Second, the comparison of the randomized treated patients to the randomized control as if they had remained on placebo.

6.4.2.1 Analysis Methodology Summary

Datametrics Research (Professor Donald B. Rubin) will perform the analysis of the data. Genzyme will be responsible for providing the necessary analysis data sets to Datametrics Research. The analysis will be done in three stages.

First, the historical patients will be matched to the patients in the randomized study. The matching will be performed by Datametrics Research without any information corresponding to patient’ outcome data in either the historical data set or the randomized experiment. Datametrics Research will initially receive only the baseline characteristics of the patients; when the matching is complete, the outcome data for the historical controls that were chosen to be matched will be provided for the second stage.

The second stage will multiply impute the missing data, both background and outcome, in the historical control data set for the set of units chosen to be matched to the 76 patients in the Phase 4 Study. In this stage, no data from those randomized to Fabrazyme® will be available to Datametrics Research. Because the outcome data in the historical controls are relatively sparse, Datametrics Research will use models of the outcome data for the placebo controls to help create the imputations in the historical controls. Also, the historical controls will be used to help impute the post-open-label data for the placebo controls as if they were still on placebo.

Only after the first two stages are fully complete will the third stage begin: the analysis and comparison of outcome data from the matched historical controls and the randomized

experiment. This stage will be the first time any outcome data from the patients randomized to Fabrazyme® will be available to Datametrics Research.

Stage 1: Matching Algorithm

The objective of matching the patients in the historical study to patients in the randomized study is to minimize the bias between the groups by ensuring balance between the randomized patients and the corresponding control group relative to a set of observed covariates.

Propensity scoring algorithms following the method described in Rubin (*Rubin, 2001, Health Serv & Outcomes Res Meth*) will be utilized to match the historical patients to the randomized patients. The end results will be a subset of historical patients, which are as comparable as possible to the set of randomized patients. Table 6-4 lists the covariates considered in the generation of propensity scores. The matching algorithm is such that greater emphasis is given to matching on the gender, age, and baseline serum creatinine covariates.

Table 6-4 List of Observed Covariates Used in the Matching Algorithm

Baseline Serum Creatinine	Baseline GFR
Age	Estimated Creatinine Clearance
Gender	Race
Blood Group {A+,A-,O+,O-}, {B+, B-,AB+, AB-}	Plasma a-GAL
Height	Weight

Subclassification of the matched patients will be used to create sub-groups of matched patients based on the propensity scores (*Rubin, 2001, Health Serv & Outcomes Res Meth*).

Subclassification provides further balance between the two patient groups with respect to the observed covariates, and is especially important when the control group is not many times larger than the treated group.

Stage 2: Multiple Imputation

At this point, there will exist two control groups for those randomized to Fabrazyme®: the placebo controls and the historical controls. Each has a missing data problem. After open-label, the placebo controls are missing their outcomes under continued placebo. The historical controls are missing many of the measurements taken in the randomized groups. Both sets of missing data will be multiply imputed following the broad guidelines in Rubin (*Rubin, 1987, in Multiple*

Imputation for Non-response in Survey). At no point in this imputation will the outcome data from the randomized treated group be available to Datametrics Research.

The imputation of missing data for the two control groups will be done by utilizing data from each other. The placebo controls providing detailed measurements of serum creatinine progression in the absence of treatment in the short term, and the historical controls providing measurements of the longer term progression in a larger group.

Stage 3: Analysis of Randomized Patients to Matched Historical Patients

At the end of the second stage, there will be a Fabrazyme® treated group of approximately 50, a chosen historical control group of approximately 85, and a placebo control group of approximately 25. Each set of multiple imputations will create one "complete" data set, with serum creatinine levels for patients treated with Fabrazyme® and the corresponding imputed and actual serum creatinine levels for the controls. Each such data set will be analyzed as specified, and the results combined using the standard multiple imputation combining rules to generate one inference for each analysis.

For example, in each of the multiply imputed data sets, the predicted number of events in the treated group will be calculated as if they were untreated and thereby determine a distribution of the number of events that reflects sampling variability. This determination may be used to perform a number of analyses such as: an interval estimate or hypothesis test; calculation of the time that each patient is predicted to be event-free when untreated; Kaplan-Meier analysis. Genzyme and FDA will agree on the finalization of this stage before outcome data from the treated patients are seen.

6.5 Phase 4 Summary

In accordance with the Accelerated Approval regulations, Genzyme has initiated an adequate and well-controlled post-marketing study designed to verify and describe the clinical benefit inferred from the very robust results obtained employing a mutually agreed upon surrogate endpoint in the Phase 3 study. Indeed the Phase 4 study has been ongoing for almost 2 years and is fully enrolled. Additionally, an extensive 447 Fabry patient historical database has been developed using a prospectively defined protocol. This database allows for more accurate determinations of event rate and sample size estimates for the Phase 4 study, and provides an opportunity to create historical control groups of patients with Fabry disease that fit the inclusion and exclusion criteria of ongoing and future Genzyme studies with Fabrazyme. In order to address concerns regarding the ethics and feasibility of conducting a placebo-controlled study in a post-marketing setting in which the endpoint will likely result in irreversible end organ damage, Genzyme has proposed converting the placebo-controlled Phase 4 study to a carefully controlled historical control study. In addition, the Phase 4 program would be expanded to include comparing the patients in the Phase 3 Extension study, who have less advanced renal disease than the patients in

the Phase 4 study, with an appropriate historical control group. Several distinct and credible statistical methodologies exist which may be employed to (1) derive appropriate historical control groups from the 447 Fabry Natural History Database, and (2) compare the historical control group to the active treatment group with respect to renal event rates.

Each of the Phase 4 trial designs presented have advantages and disadvantages. The placebo-controlled trial design limits the population of Fabry patients that can be studied in a reasonable timeframe to those with some degree of renal insufficiency at baseline and raises ethical and feasibility concerns in a post-marketing setting. The historical control designs allow for a broader cross section of Fabry patients to be studied and obviate the ethical and feasibility concerns. However, the historical control designs raise questions regarding comparability of the groups. The statistical methodology proposed here addresses this important issue. Therefore, Genzyme believes that when one carefully balances the advantages and disadvantages of each approach, employing an historical control design is most appropriate. Most importantly, the vast amount of data that has already been generated in this ultra orphan disease allows these several different, and not mutually exclusive, options to be considered and pursued in the post-approval setting to demonstrate the long-term clinical benefit of Fabrazyme. Thus, the requirements under accelerated approval for adequate and well-controlled post-marketing studies to be underway at the time of approval of the BLA have been met. Indeed, the final details around the method of analysis to be employed for assessing the long-term clinical benefit of Fabrazyme compared to a natural history control group can be established in the post-approval setting so that patients can be allowed access to a therapy for their Fabry disease.

7. CONCLUSION

Currently, no treatment is available for the prevention or stabilization of the progressive vascular damage and resultant end organ destruction of Fabry disease. The GL3 accumulation in multiple cell types, but particularly the endothelial cells, leads to the high morbidity and mortality rates due to renal failure, stroke, and cardiovascular disease.

Fabrazyme therapy has been shown to clear GL-3 to normal or near-normal levels in critical cells involved in the pathophysiology of the disease, including the capillary endothelium, and thus has the potential for long-term clinical benefit. This is further supported by trends in patients receiving Fabrazyme for up to 30 months where data indicate a slowing in progression of renal functional decline compared to a matched historical database of untreated Fabry patients. The data demonstrate that Fabrazyme is well-tolerated and has a robust efficacy profile.

The ongoing Phase 4 study (fully enrolled, with the first patient having received treatment for approximately 21 months) can now be converted to an historical control study based on the significant historical data that have been collected. This, along with the additional aspects of the proposed expanded Phase 4 program, ensure that data will be collected in the post-marketing setting to demonstrate the long-term clinical benefit of Fabrazyme. Thus the requirements under accelerated approval for adequate and well-controlled post-marketing studies to be underway at the time of approval of the BLA have been met. At this time, the data available indicate that Fabrazyme is effective treatment for patients with Fabry disease. Therefore, patients should not be denied the opportunity of this therapy, and approval is warranted at this time.

- 8. APPENDICES**
- 8.1 Proposed Phase 4 Single Arm Protocol Synopsis**
- 8.2 Published Results of Clinical Studies with Fabrazyme**
- 8.3 Final Report: Epidemiological Study of Fabry Disease**
- 8.4 Statistical Analysis Plan: Matched Historical Control Methodology**

Genzyme Corporation
One Kendall Square
Cambridge, MA 02139
USA
(617) 252-7600

Genzyme Europe B.V.
Gooimeer 10
1411 DD Naarden
The Netherlands
+31 35 699 1200

Study Number AGAL-008-00

Multi-center Active Treatment Study of the Safety and Efficacy of Recombinant Human α -Galactosidase A (r-h α GAL) on Progression of Renal Disease in Patients with Fabry Disease Compared to an Historical Cohort of Untreated Patients with Fabry Disease

Final: 27 September 2000
Amendment 1: 16 November 2000
Amendment 2: 5 December 2000
Amendment 3: 13 April 2001
Amendment 4: 14 March 2002
Amendment 5: TBD

Study Director: Donna Mackey
Associate Director, Clinical Research
Genzyme Corporation
(617) 591-5859

Medical Monitor: Rekha Abichandani, MD
Associate Medical Director, Clinical Research
Genzyme Corporation
(617) 591-5741

Statistician: Melissa Nichols, MS
Senior Biostatistician
Genzyme Corporation
(617) 591-7082

This protocol was designed and will be conducted, recorded, and reported in compliance with the principles of Good Clinical Practice (GCP) guidelines. These guidelines are stated in U.S. federal regulations as well as "Guidance for Good Clinical Practice," International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

I have read and agree to abide by the requirements of this protocol.

Investigator Signature

Date

1. SYNOPSIS

<p>NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139</p> <p>NAME OF FINISHED PRODUCT Fabrazyme™</p> <p>NAME OF ACTIVE INGREDIENT Recombinant Human α-galactosidase A</p>	<p>SUMMARY TABLE</p> <p>Referring to Part of the Dossier:</p> <p>Volume:</p> <p>Page:</p> <p>Reference:</p>	<p>FOR NATIONAL AUTHORITY USE ONLY:</p>
<p>TITLE</p> <p>Multi-center Active Treatment Study of the Safety and Efficacy of Recombinant Human α-Galactosidase A (r-hαGAL) on Progression of Renal Disease in Patients with Fabry Disease Compared to an Historical Cohort of Untreated Patients with Fabry Disease</p>		
<p>PROTOCOL NO.</p> <p>AGAL-008-00</p>		
<p>INVESTIGATOR/ STUDY CENTERS</p> <p>Approximately 30 study centers worldwide will participate in this clinical trial.</p>		
<p>OBJECTIVES</p> <p>The primary objective of this study is to assess the effectiveness of r-hαGAL by showing a statistically significant decrease in the proportion of patients who have a renal event defined by a 50% increase in serum creatinine levels or progression to end stage renal disease (need for chronic dialysis and/or kidney transplantation), as compared to the estimated population proportion from a well-characterized historical cohort of patients with advanced Fabry disease who have not received treatment with enzyme replacement therapy.</p>		
<p>METHODOLOGY</p> <p>This will be a multicenter, multinational, historical control trial to assess the safety and effectiveness of r-hαGAL in patients with advanced Fabry disease receiving r-hαGAL therapy as treatment for Fabry disease compared to a well-characterized historical cohort of patients with Fabry disease who have not received treatment with enzyme replacement therapy.</p> <p>All patients enrolled in this study will receive treatment with r-hαGAL.</p> <p>This study was originally placebo-controlled. Upon approval of Amendment 5 and the resulting conversion to active treatment, the blind was broken and all patients were converted to active treatment with r-hαGAL.</p> <p>Approximately 70 patients will be enrolled in the active treatment arm at approximately 30 study centers. Patients will receive approximately 1 mg/kg (0.9 to 1.1 mg/kg) of r-hαGAL every 2 weeks for at least 36 months. The study will conclude 36 months after the last patient begins active treatment with r-hαGAL.</p>		
<p>NUMBER OF SUBJECTS</p> <p>Approximately 70 patients will be enrolled.</p>		
<p>DIAGNOSIS/INCLUSION CRITERIA</p> <p>The patient must provide written informed consent prior to any study-related procedures being performed, be</p>		

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Fabrazyme™ NAME OF ACTIVE INGREDIENT Recombinant Human α-galactosidase A	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
<p>≥ 16 years old, have a current diagnosis of Fabry disease; patients may not have received enzyme replacement therapy as treatment for Fabry disease; patients must have documented plasma α-galactosidase A (αGAL) activity of < 1.5 nmol/hr/mL or a documented leukocyte αGAL activity of < 4 nmol/hr/mg. Patients must have a clinical presentation consistent with Fabry disease (e.g., angiokeratoma, Fabry pain, decreased sweating, corneal opacities, etc.). Patients must have one or more of the following: a serum creatinine measurement of 1.2 to 3 mg/dL (106.1 to 265.2 μmol/L) OR estimated creatinine clearance < 80 mL/min only if the patient's serum creatinine measurement < 1.2 mg/dL.</p> <p>Female patients of childbearing potential must have a negative pregnancy test (urine β-hCG) prior to each dosing; in addition, all female patients of childbearing potential must use a medically accepted method of contraception throughout the study. Patients must have the ability to comply with the clinical protocol.</p> <p>A patient will not be eligible for the study if: Patient has undergone or is currently scheduled for kidney transplantation or is currently on dialysis; has acute renal failure; has received prior enzyme replacement therapy as treatment for Fabry disease; has participated in a study employing an investigational drug within 30 days of the start of their participation in this trial; has diabetes mellitus or presence of confounding renal disease; has history of TIA or ischemic stroke within 3 months of study entry documented by mild-to-moderate neurological deficit; has current critical coronary artery disease (as documented by a presently unstable angina and/or documented myocardial infarction within 3 months); has congestive heart failure (contributed by Fabry disease) as defined by Class III or Class IV cardiac status as evaluated under the New York Heart Association classification; has severe residual neurological deficit that will confound the detection of new events as determined by an attending neurologist and/or Principal Investigator; has a clinically significant organic disease or an unstable condition that, in the opinion of the Investigator, would preclude participation in the trial; is pregnant or lactating; is unwilling to comply with the requirements of the protocol; or has a medical condition, serious intercurrent illness, or extenuating circumstance that would significantly decrease study compliance, including prescribed follow-up.</p>		
<p>DOSE/ROUTE/REGIMEN</p> <p>Patients will receive approximately 1 mg/kg (0.9 to 1.1 mg/kg) r-hαGAL intravenously every 2 weeks for approximately 36 months. The study will conclude 36 months after the last patient begins active treatment with r-hαGAL. The infusion will be administered via IV pump at a rate of no more than 0.25 mg/min (15 mg/hr) over approximately 4 – 6 hours for the first 8 infusions. After the eighth infusion, the infusion rate may be increased, as tolerated; however, the total infusion period may not be less than 2 hours. Those patients previously randomized to the placebo arm of the study will receive their first 8 infusions of active treatment at a rate of no more than 0.25 mg/min (15 mg/hr) over approximately 4 – 6 hours. After the eighth infusion, the infusion rate may be increased, as tolerated; however, the total infusion period may not be less than 2 hours.</p>		
<p>REFERENCE TREATMENT</p> <p>There will be no reference treatment.</p>		
<p>STUDY DURATION</p>		

GENZYME CORPORATION

FINAL: 27 SEPTEMBER 2000
 AMENDMENT 1: 16 NOVEMBER 2000
 AMENDMENT 2: 5 DECEMBER 2000
 AMENDMENT 3: 13 APRIL 2001
 AMENDMENT 4: 14 MARCH 2002
 AMENDMENT 5: TBD

<p>NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139</p> <p>NAME OF FINISHED PRODUCT Fabrazyme™</p> <p>NAME OF ACTIVE INGREDIENT Recombinant Human α-galactosidase A</p>	<p>SUMMARY TABLE</p> <p>Referring to Part of the Dossier:</p> <p>Volume:</p> <p>Page:</p> <p>Reference:</p>	<p>FOR NATIONAL AUTHORITY USE ONLY:</p>
<p>The study duration is approximately 36 months. The study will conclude 36 months after the last patient begins active treatment with r-hαGAL.</p>		
<p>CRITERIA FOR EVALUATION</p> <p>Efficacy: Effectiveness will be evaluated by showing a statistically significant decrease in the proportion of patients who have a renal event defined by a 50% increase in serum creatinine levels or progression to end stage renal disease (need for chronic dialysis and/or kidney transplantation), as compared to the estimated population proportion from a well-characterized historical cohort of patients with advanced Fabry disease who have not received treatment with enzyme replacement therapy. Where available, the pre-treatment 1/serum creatinine slopes will be compared to the post-treatment 1/serum creatinine slopes of each treated patient in order to assess within-group differences.</p> <p>Safety: Safety will be measured in terms of adverse experiences, vital sign parameters, electrocardiogram (ECG) parameters, echocardiograms, physical examinations, and laboratory safety parameters.</p>		
<p>STATISTICAL METHODS</p> <p>Efficacy analyses will be performed on all enrolled patients (Intent-to-treat population) as well as a per protocol population. Protocol violations will be prospectively defined in the statistical analysis plan. Any patient missing 20% or more of the infusions will be excluded from the per protocol population.</p> <p>For the primary endpoint, an estimate of the proportion of renal events occurring within three years for treated patients will be calculated using a Kaplan-Meier estimate. Then a one sample, two-tailed binomial confidence interval will be derived to estimate with 95% confidence, the boundaries in which the population parameter exists for treated patients (actual proportion of treated patients with renal events) lies, given the population parameter for the untreated patients is fixed at the proportion of renal events defined from the historical patients.</p> <p>For the secondary endpoints, Predicted GFR will be calculated for each serum creatinine value collected from infusion 1 onward for treated patients and start date onward for historical patients to final study visit or event visit (if applicable). Progression of renal disease will be measured by the slope of the reciprocal of the serum creatinine concentration (i.e., 1/serum creatinine)^{Error! Bookmark not defined.} for both patient groups. The slope will be estimated from infusion 1 onward for the treated patients and start date onward for the historical patients. For both predicted GFR and 1/serum creatinine, the distribution of the slopes will be compared between the two patient groups using a Wilcoxon Rank-Sum Test.</p> <p>Predicted glomerular filtration rate (GFR) using the Modification of Diet in Renal Disease (MDRD) Study Group equation:^{Error! Bookmark not defined.}</p> $\text{Predicted GFR (mL/min/1.73 m}^2\text{)} = 186 \times (\text{PCr})^{-1.154} \times (\text{age in years})^{-0.203} \times (0.742, \text{ if patient is female}) \times (1.212, \text{ if patient is black})$ <p>where PCr is serum creatinine measured in mg/dL.</p> <p>Predicted GFR and 1/serum creatinine will also be categorized as tertiary endpoints, but only for the treated patients. Historical serum creatinine values were collected for treated patients prior to the administration of</p>		

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Fabrazyme™ NAME OF ACTIVE INGREDIENT Recombinant Human α-galactosidase A	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
<p>drug. Predicted GFR and 1/serum creatinine will be calculated for all historical and post-treatment serum creatinine values. A slope of these historical values will be derived and a slope of the post treatment values will be derived for each patient. The distribution of these slopes will be compared to zero using a Wilcoxon signed-rank test. Plasma GL-3, proteinuria defined by the ratio of urinary protein to urinary creatinine, and proteinuria defined by the ratio of urinary micro-albumin to urinary creatinine are also tertiary endpoints. The change from pre-treatment to post-treatment will be calculated for each and then the distribution of change will be analyzed using a Wilcoxon signed rank test. The proportion of patients that have Cerebral Vascular disease events, cardiac events and death will be summarized for the treated patients and the historical patients. No formal statistical comparisons will be conducted on these parameters as there are limitations to the collection of these events for the historical patients. The continuous parameters collected from the echocardiogram will be analyzed for the treated patients using a Wilcoxon signed-rank test. For discrete parameters for the echocardiogram, a one sample binomial test will be used for treated patients only.</p> <p>Safety analyses will be performed on the Intent-To-Treat (ITT) population. Vital signs, 12-lead ECGs, echocardiograms, antibodies, physical exams and laboratory evaluations will be summarized for treated patients. Laboratory evaluations, physical exams and medical history will be summarized for the historical patients. Summary statistics will be computed for the safety variables at each visit that they are collected at as well as the changes from Visit 1 (Day 0) to final study visit or event. Adverse experiences will be coded using the WHO ART dictionary. A detailed listing of patients who experience adverse events and serious adverse events will be presented.</p>		

A Phase 1/2 Clinical Trial of Enzyme Replacement in Fabry Disease: Pharmacokinetic, Substrate Clearance, and Safety Studies

Christine M. Eng,^{1,*} Maryam Banikazemi,¹ Ronald E. Gordon,² Martin Goldman,³ Robert Phelps,⁴ Leona Kim,³ Alan Gass,³ Jonathan Winston,³ Steven Dikman,² John T. Fallon,^{2,3} Scott Brodie,⁵ Charles B. Stacy,⁶ Davendra Mehta,³ Rosaleen Parsons,⁷ Karen Norton,⁷ Michael O'Callaghan,⁸ and Robert J. Desnick¹

Departments of ¹Human Genetics, ²Pathology, ³Medicine, ⁴Dermatology, ⁵Ophthalmology, ⁶Neurology, and ⁷Radiology, Mount Sinai School of Medicine, New York, and ⁸Genzyme Corporation, Cambridge, MA

Fabry disease results from deficient α -galactosidase A (α -Gal A) activity and the pathologic accumulation of the globotriaosylceramide (GL-3) and related glycosphingolipids, primarily in vascular endothelial lysosomes. Treatment is currently palliative, and affected patients generally die in their 40s or 50s. Preclinical studies of recombinant human α -Gal A (r-h α GalA) infusions in knockout mice demonstrated reduction of GL-3 in tissues and plasma, providing rationale for a phase 1/2 clinical trial. Here, we report a single-center, open-label, dose-ranging study of r-h α GalA treatment in 15 patients, each of whom received five infusions at one of five dose regimens. Intravenously administered r-h α GalA was cleared from the circulation in a dose-dependent manner, via both saturable and non-saturable pathways. Rapid and marked reductions in plasma and tissue GL-3 were observed biochemically, histologically, and/or ultrastructurally. Clearance of plasma GL-3 was dose-dependent. In patients with pre- and posttreatment biopsies, mean GL-3 content decreased 84% in liver ($n = 13$), was markedly reduced in kidney in four of five patients, and after five doses was modestly lowered in the endomyocardium of four of seven patients. GL-3 deposits were cleared to near normal or were markedly reduced in the vascular endothelium of liver, skin, heart, and kidney, on the basis of light- and electron-microscopic evaluation. In addition, patients reported less pain, increased ability to sweat, and improved quality-of-life measures. Infusions were well tolerated; four patients experienced mild-to-moderate reactions, suggestive of hypersensitivity, that were managed conservatively. Of 15 patients, 8 (53%) developed IgG antibodies to r-h α GalA; however, the antibodies were not neutralizing, as indicated by unchanged pharmacokinetic values for infusions 1 and 5. This study provides the basis for a phase 3 trial of enzyme-replacement therapy for Fabry disease.

Introduction

Fabry disease (MIM 301500) is an X-linked inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal exoglycohydrolase, α -galactosidase A (α -Gal A) (Desnick et al. 1995, 2001). In patients with the classical disease phenotype, the progressive accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids, particularly in vascular endothelial lysosomes in the heart, liver, kidney, skin, and brain, leads to the major disease manifestations. Clinical onset occurs in childhood or adolescence and is

characterized by severe acroparesthesias, angiokeratoma, corneal and lenticular opacities, and hypohidrosis. With advancing age, renal failure and vascular disease of the heart and brain lead to early demise, the average age at death being 41 years in one series (Colombi et al. 1967). To date, there is no specific therapy for Fabry disease, and treatment is supportive, limited to symptomatic management of the acroparesthesias and episodes of excruciating pain and of complications of renal failure, cardiac, or cerebrovascular disease.

It is of relevance that patients with residual α -Gal A activity (~1% to ~10% of normal) are essentially asymptomatic or have a mild form of the disease limited to cardiac involvement (von Scheidt et al. 1991; Desnick et al. 2001). These "cardiac variants" typically present in late adulthood (>40 years) with left ventricular hypertrophy, cardiomyopathy, and/or mild proteinuria. They lack the classic disease manifestations, which include angiokeratoma, acroparesthesias, hypohidrosis, corneal/lenticular dystrophy, and renal failure. A consistent feature of the cardiac variant is the absence of

Received December 27, 2000; accepted for publication January 12, 2001; electronically published February 1, 2001.

Address for correspondence and reprints: Dr. R. J. Desnick, Professor and Chairman, Department of Human Genetics, Box 1498, Mount Sinai School of Medicine, Fifth Avenue and 100th Street, New York, NY 10029. E-mail: rjdesnick@mssm.edu

* Present affiliation: Department of Molecular and Human Genetics, Baylor College of Medicine, Houston.

© 2001 by The American Society of Human Genetics. All rights reserved.
0002-9297/2001/6803-0016\$02.00

lysosomal glycosphingolipid accumulation in the vascular endothelium (Elleder et al. 1990; von Scheidt et al. 1991), the pathogenic hallmark of classically affected patients (Desnick et al. 2001). The cardiac variants demonstrate that even low levels of α -Gal A activity can markedly alter the classic disease phenotype, particularly the morbidity associated with the progressive vascular endothelial glycosphingolipid deposition.

Early pilot trials of enzyme replacement in classically affected males involved the intravenous administration of either a single dose of fresh normal plasma containing active α -Gal A (Mapes et al. 1970) or a single dose of the partially purified placental enzyme (Brady et al. 1973). These studies demonstrated that intravenously infused enzyme from plasma or placenta could decrease the level of accumulated plasma GL-3. In a subsequent study, two affected brothers each received six doses of α -Gal A purified from splenic tissue or plasma over a 3-mo period (Desnick et al. 1979). The splenic-derived enzyme was cleared rapidly from the circulation ($t_{1/2}$ ~10 min), transiently decreasing the circulating GL-3 concentration, whereas the more highly sialylated plasma-derived enzyme had a slower clearance ($t_{1/2}$ ~70 min) and effected a longer depletion of circulating GL-3. Notably, two doses of the plasma-derived enzyme, administered on days 1 and 3, reduced the plasma substrate level into the normal range (Desnick et al. 1980). These studies demonstrated the feasibility of enzyme-replacement therapy for Fabry disease; however, the difficulty of producing enough purified enzyme for clinical trials was a major obstacle.

This obstacle was overcome following the isolation of the human α -Gal A cDNA (Bishop et al. 1986) and demonstration of its high-level expression in Chinese hamster ovary (CHO) cells (Ioannou et al. 1992), thereby providing a source of both lysosomal (oligosaccharide processed) and secreted (highly-sialylated) glycoforms of the enzyme (Matsuura et al. 1998). In addition, the generation of knockout "Fabry mice" with α -Gal A deficiency (Wang et al. 1996) permitted determination, by preclinical studies, of the pharmacokinetics and biodistributions of various recombinant human α -Gal A (r-h α GalA) glycoforms (Ioannou et al. 2001). When a highly sialylated glycoform (AGA-1) was used, these animal-model studies demonstrated a dose-dependent clearance of accumulated GL-3 from the circulation and from the pathologic sites of substrate deposition in the liver, heart, kidney, and skin (Ioannou et al. 2001), thereby establishing "proof of concept" for clinical trials of r-h α GalA replacement in patients with Fabry disease. In addition, in a recent phase 1 study, single doses of recombinant enzyme reduced GL-3 levels in liver and urinary sediment (Schiffmann et al. 2000).

Here, we report the results of a phase 1/2 trial of five dose regimens of r-h α Gal A in 15 classically affected

males with Fabry disease. This open-label dose-escalation study was conducted to evaluate the safety and pharmacokinetics of r-h α GalA infusions and to provide preliminary efficacy data for enzyme-replacement therapy in this lysosomal-storage disease.

Material and Methods

Study Design

Fifteen classically affected male patients with Fabry disease were recruited for this multidose, open-label, single-center, dose-escalation study of r-h α GalA (Fabrazyme [agalsidase beta]; Genzyme). Participants were aged ≥ 16 years and had α -Gal A activity in plasma < 1.5 nmol/h/ml, GL-3 concentrations in plasma ≥ 5.0 ng/ μ l, serum creatinine < 2.5 mg/dl, and no history of renal dialysis or transplantation. Patients were sequentially enrolled into one of five r-h α GalA dosing regimens with three patients per group. The characteristics of the five groups are shown in table 1. Patients in each group received five doses of enzyme as follows: Group A, 0.3 mg/kg of r-h α GalA every 14 d (biweekly); Group B, 1.0 mg/kg biweekly; Group C, 3.0 mg/kg biweekly; Group D, 1.0 mg/kg every 48 h; and Group E, 3.0 mg/kg every 48 h. R-h α GalA was synthesized in CHO cells, and the secreted homodimeric enzyme was purified from the growth medium. Each enzyme subunit contained three glycosylation sites, with the predominant oligosaccharides being bi- and monophosphorylated oligomannose structures and sialylated complex structures. Enzyme was diluted to 100 ml with saline, and infusions were given intravenously at a rate of 0.83 ml/min. The institutional review board of the Mount Sinai School of Medicine approved the study, and all patients gave written informed consent to participate in the study.

Clinical Assessments

Medical and safety evaluations—including medical history, physical examinations, vital signs, routine serum and urine chemistries, hematology indices, and EKGs—were performed at baseline, prior to each infusion, and after infusion 5. In addition, echocardi-

Table 1

Summary of Patient Demographics

DOSE REGIMEN	MEAN AGE (RANGE) (years)	MEAN WEIGHT (RANGE) (kg)	RACE (n)	
			White	Hispanic
0.3 mg/kg/14 d	41.0 (35–44)	64.7 (57–71)	3	0
1.0 mg/kg/14 d	33.7 (27–38)	73.6 (57–88)	1	2
3.0 mg/kg/14 d	34.7 (32–37)	69.1 (56–82)	2	1
1.0 mg/kg/48 h	27.0 (18–37)	69.8 (65–73)	2	1
3.0 mg/kg/48 h	35.7 (30–45)	78.0 (67–93)	3	0

ograms and renal magnetic resonance imaging (MRI) were evaluated at baseline and after infusion 5. Patients completed the Short Form McGill Pain Survey (Melzack 1987) and the Short Form-36 (SF-36) Health Survey (Ware et al. 1997) to assess quality-of-life measures at baseline and after infusion 5. Patients continued their usual prophylactic and analgesic pain medications. Adverse events were coded using the World Health Organization Adverse Reactions Thesaurus (WHOART). At baseline, prior to each infusion, and after infusion 5, an enzyme-linked immunosorbent assay (ELISA), specific for r-h α GalA, and a confirmatory radioimmunoprecipitation technique were used to assess antibody response to r-h α GalA.

Molecular and Biochemical Studies

Specific mutations in the α -Gal A gene in each patient were previously determined (e.g., Eng et al. 1993). Plasma and tissue α -Gal A activities were measured using 2.5 mM 4-methylumbelliferyl- α -D-galactoside as substrate (Desnick et al. 1973). Plasma and tissue GL-3 concentrations were measured using a verotoxin-based ELISA specific for GL-3 (Zeidner et al. 1999). Plasma GL-3 levels were measured at baseline, before each infusion, and either 14 d (for the every-48-h groups) or 21–28 d (for the biweekly groups) after infusion 5.

Collection of Tissue Samples

Percutaneous needle biopsies of liver and 3-mm punch biopsies of skin from the lower back (avoiding angiokeratomas) were collected from all patients at baseline and 2–3 d after infusions 1 and 5. Pre- and posttreatment right-ventricular endomyocardial and kidney biopsies were optional procedures for a subset of participants in groups C, D, and E only. Tissue samples were divided and then prepared for light and electron microscopy or frozen at -80°C for biochemical analyses.

Pharmacokinetic Analyses

For infusions 1 and 5, r-h α GalA activity was determined from blood samples collected at 30, 60, and 90 min during the infusions and at multiple time points 0–480 min after these infusions. These data were evaluated using standard noncompartmental analysis. In addition, concentration-time data were subjected to model-independent pharmacokinetic analysis. The following pharmacokinetic values were determined: (1) area under the curve ($\text{AUC}_{0-\infty}$), as computed by the linear trapezoidal rule; (2) area under the moment ($\text{AUMC}_{0-\infty}$); (3) peak α -Gal A concentration (C_{max}); (4) time to peak activity (T_{max}); (5) the terminal elimination half-life ($t_{1/2}$); (6) volume of distribution at steady state (VSS); (7) clearance; (8) mean residence time extrapolated to

infinity (MRT_{∞}); and (9) the elimination-rate constant (λ_z).

Histological Evaluations

For light microscopy, tissue was embedded in paraffin (liver, kidney, and skin), glycomethacrylate (kidney), or Epon (skin and heart). Sections were stained with hematoxylin and eosin (liver, kidney), periodic acid-Schiff (liver, skin, and kidney), methylene blue/azure II (skin, heart, kidney), and/or oil red O (skin). For electron microscopy, gluteraldehyde-fixed tissue was posttreated with OsO_4 , was epoxy-embedded, was sectioned (4–5 μm), and was viewed under a JOEL transmission electron microscope. Representative tissue specimens were photographed. Scoring of tissue response to treatment was performed with coded instruments designed by expert pathologists for light- and electron-microscopic assessment of the tissue and cell types with significant GL-3 accumulation. Quantitation of GL-3 content for both light and electron microscopy was based on a 4-point scoring system to evaluate the degree and extent of glycosphingolipid inclusions (ranging from 0, normal or near normal, to 3, severe involvement). This system was applied to several structures in each tissue. Exceptions to this format were an extended coding range for the vascular endothelium of the skin (0–5) to account for any accidental inclusion of angiokeratomatous vessels in the biopsy, and a histomorphometric method applied to the tightly grouped inclusions in cardiomyocytes with scores expressed as the volume of inclusions relative to the total volume of tissue evaluated.

Statistical Analyses

Statistical analyses were performed using the Statistical Software System (SAS Institute). The SF-36 questionnaire was analyzed using the SF-36 health scoring system (Ware et al. 1997). A Wilcoxon signed rank test was used to calculate change from baseline for quality of life and pain (Short Form McGill Pain Questionnaire) values (Melzack 1987).

Results

Pharmacokinetics

Concentration-time data for r-h α GalA infusions demonstrated a dose-dependent (nonlinear) profile consistent with enzyme clearance from the circulation via both saturable and nonsaturable (concentration-independent) pathways. Semilog plots of mean concentration-time data for the first 2 h of infusion of r-h α GalA at doses of 0.3, 1.0, or 3.0 mg/kg biweekly (groups A–C) are shown in figure 1. Mean plasma concentrations reached 80% of peak values 60 min into the infusion for the 0.3 mg/kg dose (group A) and 90 min into the infusion for

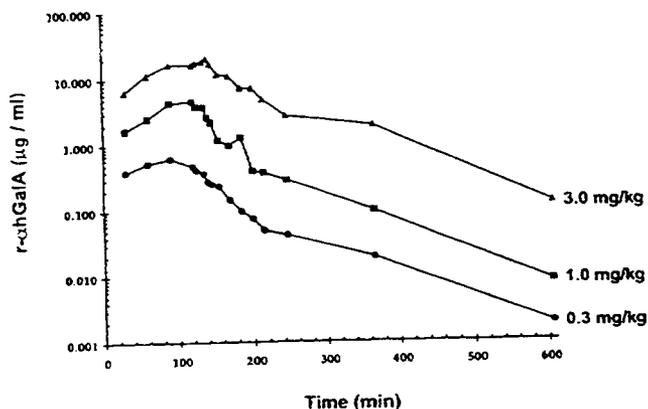


Figure 1 Pharmacokinetics of r-h α GalA infusions. Semilog plots of mean concentration-time data for r-h α GalA infusions at doses of 0.3 (\blacklozenge), 1.0 (\bullet), or 3.0 (\blacktriangle) mg/kg (groups A-C), demonstrating the dose-dependent (nonlinear) profile consistent with enzyme cleared from the circulation via both saturable and nonsaturable (concentration independent) pathways. R-h α GalA was infused over 120 min. See text for details.

the 1.0 and 3.0 mg/kg doses (groups B and C). When infusions were completed, mean concentrations dropped to half-peak values within 15, 20, and 45 min for the 0.3-, 1.0-, and 3.0-mg/kg dose groups, respectively. Clearance appeared to be biphasic for all biweekly dose groups, with the more rapid elimination phase lasting 1-2 h after infusion. AUC values were increased disproportionately with dose, from ~ 80 to ~ 500 to ~ 4000 $\mu\text{g}/\text{min}/\text{ml}$. The mean VSS had a range of 80-330 ml/kg (1-4 times blood volume). Clearance decreased from 4 ml/min/kg to ~ 1 ml/min/kg with increasing dose. Of note, the terminal elimination half-life was not affected by dose, which is consistent with elimination being governed, in part, by a first-order, concentration-independent mechanism.

Plasma GL-3 Clearance was Dose-Dependent

Plasma GL-3 concentrations were reduced in a dose-dependent manner for all infusion groups (fig. 2). Prior to treatment, all patients had elevated plasma GL-3 levels, with a range of 2.0-53.9 ng/ μl (mean 17.1 ± 12.8 ng/ μl); the normal, undetectable level is <1.2 ng/ μl). Plasma GL-3 levels in patients receiving r-h α GalA at 0.3 mg/kg biweekly tended to decrease with each dose, reaching their lowest values only at infusion 5. In contrast, plasma GL-3 levels in all three patients receiving r-h α GalA at doses of 3.0 mg/kg biweekly totally cleared after the first infusion and remained undetectable throughout the clinical trial. Two of three patients receiving r-h α GalA at 1.0 mg/kg biweekly demonstrated plasma GL-3 clearance after the first infusion, whereas

the third patient's plasma GL-3 level was reduced but never reached undetectable levels. In patients receiving 1.0 or 3.0 mg/kg every other day (groups D and E), the plasma GL-3 levels were lowest at infusion 4; however, the decreases were less than those observed in patients with biweekly dosing schedules (data not shown).

Tissue GL-3 Clearance Was Dose- and Organ-Dependent

Liver.—Hepatic GL-3 concentrations, measured by ELISA, were reduced by an average of 84% in the 13 patients who had pre- and posttreatment liver biopsies. As shown in table 2, GL-3 clearance was particularly consistent and profound (mean clearance 92%) in group C patients, who received 3.0 mg/kg biweekly. However, marked GL-3 reductions were observed in all dose cohorts. Histologic scores from electron-microscopic evaluations based on the 0-3 scale confirmed the positive response to r-h α GalA treatment, with reductions in mean sinusoid endothelial GL-3 accumulation scores from 2.40 ± 0.74 ($n = 15$) at baseline to 0.5 ± 0.52 ($n = 14$) after infusion 5. Mean Kupffer cell scores decreased from 2.80 ± 0.56 ($n = 15$) to 1.07 ± 0.27

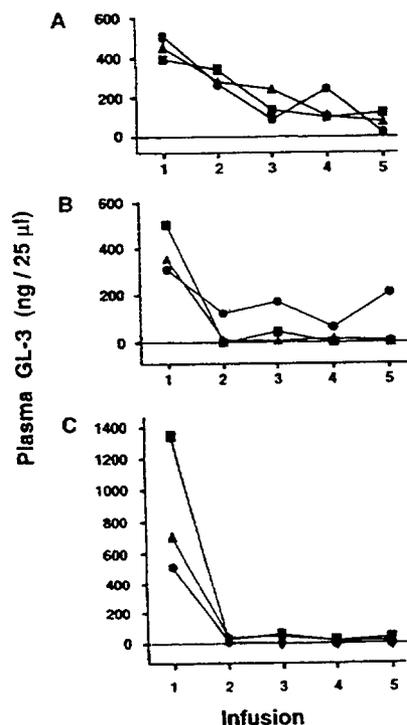


Figure 2 Effect of r-h α GalA dose on plasma GL-3 clearance. Individual patient plasma GL-3 concentrations determined just prior to infusions 1-5 for the 0.3 (A), 1.0 (B), and 3.0 (C) mg/kg biweekly dosing groups.

Table 2

GL-3 Clearance in Liver, Heart and Kidney

TISSUE, DOSE REGIMEN, AND PATIENT	AGE (years)	α -Gal A MUTATION	GL-3 CONCENTRATION		REDUCTION (%)
			at Baseline (ng/mg tissue)	2-5 d after Infusion 5	
Liver:					
Group A:					
2	44	C56G	870	74	91
3	44	N263S	1,130	48	96
Group B:					
4	27	W287C	832	185	78
5	36	C172Y	176	134	24
6	38	C172Y	2410	45	98
Group C:					
7	32	W226R	371	26	93
8	37	G138E	1,780	153	91
9	35	L89R	352	29	92
Group D:					
10	37	G138E	12,650	5,940	53
11	26	R227Q	1,280	38	97
12	18	W95S	1,640	77	95
Group E:					
13	45	1118 insT	1,690	204	88
15	32	26delA	140	0	100
Heart:					
Group C:					
7	32	W226R	17,600	15,700	11
8	37	G138E	38,100	40,900	-7
9	35	L89R	17,500	12,900	26
Group D:					
10	37	G138E	32,500	29,800	8
11	26	R227Q	17,800	14,800	17
12	18	W95S	8,320	10,700	-29
Group E:					
13	45	1118 insT	25,000	48,100	-93
Kidney:					
Group C:					
8	37	G138E	12,930	384	97
9	35	L89R	6,910	512	93
Group D:					
10	37	G138E	1,060	448	58
11	26	R227Q	10,100	1,980	80
Group E:					
13	45	1118 insT	1,860	5,630	-203

($n = 14$). A dose effect was observed for endothelial GL-3 clearance in dosing cohorts treated biweekly: from baseline, mean scores decreased -1.67 ± 0.58 points for the 0.3-mg/kg group A, -2.00 ± 1.00 points for the 1.0 mg/kg group B, and, -2.33 ± 1.15 points for the 3.0-mg/kg group C. Notably, one patient in Group E had increased sinusoid and Kupffer cell scores and was not included in the histologic analysis.

Skin.—Low and variable GL-3 levels were detected by ELISA in pretreatment skin biopsies (mean 350 ± 168 ng/mg of tissue; range 128–803 ng/mg tissue). Following infusion 5, the mean GL-3 concentration decreased

$\sim 40\%$, to 191 ± 160 ng/mg of tissue. Of the 14 patients with paired samples, 11 had reductions, and 3 (1 from group A and 2 from group D), had increases from baseline.

Pre- and posttreatment skin biopsies were obtained, free of angiokeratomas, for all patients. Histological evaluation resulted in scores of ≤ 3 on the expanded 0–5 scale, except in one patient, who had a baseline capillary endothelial score of 4 (fig. 3A). In histologic sections stained with periodic acid-Schiff, markedly reduced mean GL-3 scores were observed in the endothelium of superficial capillaries (from 2.6 ± 0.79 at baseline to

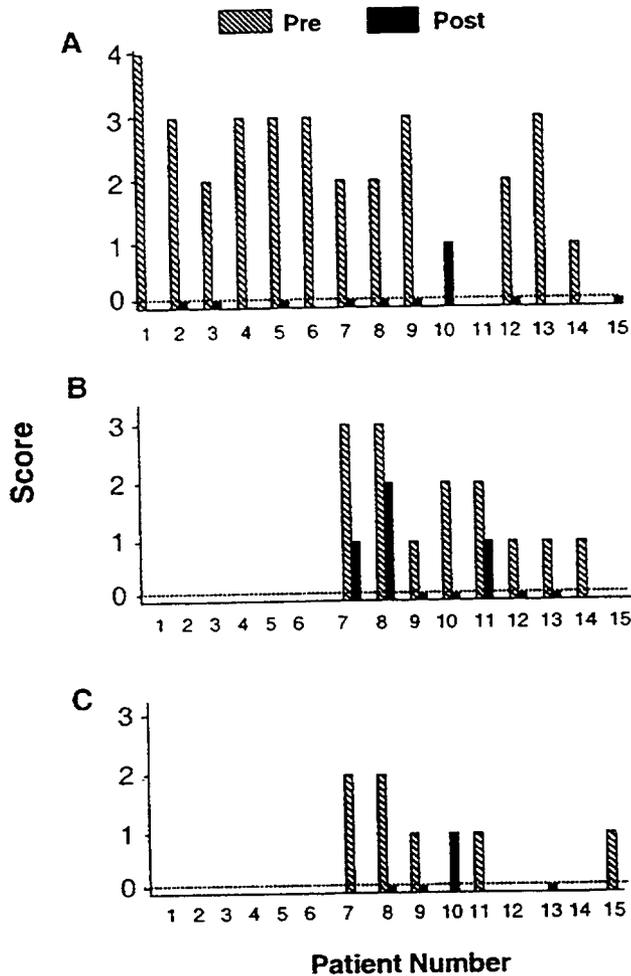


Figure 3 Light-microscopic assessment of the vascular endothelial GL-3 deposits in skin (A), heart (B), and kidney (C), before and after r-h α GalA treatment by expert pathologists blinded to time of biopsy and patient. A, Pre- and posttreatment GL-3 scores (ranging from 0, normal or near normal, to 4, severe) of skin capillary endothelium for 14 patients. Note that the seven with paired pre- and posttreatment biopsies all cleared their capillary endothelial GL-3 deposits ("0" scores). B, Pre- and posttreatment endomyocardial vascular endothelium GL-3 scores (ranging from 0, normal or near normal, to 3, severe) for seven patients: three in group C, three in group D, and one in group E. Note that the seven patients with paired pre- and posttreatment biopsies all had reduced GL-3 scores and four had cleared their capillary endothelium ("0" scores). C, Pre- and posttreatment GL-3 scores (ranging from 0, normal or near normal, to 3, severe) for kidney vascular endothelium for seven patients: three in group C, two in group D, and two in group E. Note that the two with paired pre- and posttreatment biopsies had cleared their capillary endothelial GL-3 deposits ("0" scores). See text for details.

0.11 \pm 0.33 after infusion 5), with the greatest improvement in patients in the biweekly dose cohorts. Clearance was observed with all dose regimens, best demonstrated overall by immunohistochemistry (figs. 4A and 4B). For

the perineurium, five of eight patients with paired biopsies showed decreases of 1 or 2 points from baseline. In the deeper arterioles, endothelial scores varied, with three of six patients declining by 1 or 2 points and the others remaining unchanged. Electron-microscopic evaluations provided similar results, with mean scores for GL-3 deposits in endothelial cells of superficial capillaries decreasing from 2.80 ± 0.41 at baseline to 0.50 ± 0.65 after infusion 5. Of 12 patients in treatment groups B-E, 8 essentially cleared all the accumulated GL-3 from the endothelium, achieving scores of 0 after the last infusion (e.g., figs. 4C and 4D). Similarly, mean GL-3 scores in the endothelium of deeper arterioles showed consistent decreases (3.00 ± 0.0 at baseline to 1.50 ± 1.00 ; $n = 14$). In contrast, GL-3 deposits in pericytes, perineurium, and the muscular layer of arterioles, as well as the histiocytes and fibrocytes, showed little or no change.

Heart.—Endomyocardial biopsies were obtained pre- and posttreatment in seven patients from groups C, D, and E. As shown in table 2, baseline tissue GL-3 levels, determined by ELISA, were high, with a range of 8,320–38,100 ng/mg tissue (mean $21,400 \pm 7,420$ ng/mg tissue). Following infusion 5, the mean GL-3 concentration decreased only slightly (15.6%) to $18,300 \pm 7,780$ ng/mg tissue in four patients with decreases. Histologic scores for the capillary endothelium, assessed by light microscopy (fig. 3B), decreased in all seven patients with pre- and posttreatment biopsies; mean scores decreased from 1.75 ± 0.89 at baseline to 0.57 ± 0.79 after treatment. By electron-microscopic examination (e.g., figs. 5A and 5B), six of seven paired biopsies had decreased scores for vascular endothelial GL-3, whereas one patient's scores remained unchanged (mean scores of 2.14 and 0.71, pre- and posttreatment, respectively). After five infusions, GL-3 storage remained unchanged in the cardiomyocytes, pericytes, and vascular smooth muscle.

Kidney.—Paired pre- and posttreatment kidney biopsies were obtained from five patients: two each in groups C and D, and one in group E. Pretreatment kidney GL-3 concentrations, determined by ELISA, varied considerably (table 2), with a range of 1,060–12,900 ng/mg tissue (mean $5,530 \pm 4,580$ ng/mg; $n = 5$). Following infusion 5, the mean GL-3 level was decreased by 67.6% to $1,790 \pm 2,250$ ng/mg in the five patients with paired samples. Four patients decreased their levels by a mean of 84% (mean 830 ± 770 ng/mg), whereas the fifth patient's level almost doubled. Sampling variability may account for part of this discrepancy. Histologic evaluations focused on four functional areas known to harbor prominent GL-3 inclusions: glomeruli, tubules, intertubular interstitium, and larger multilayered vessels. There was a consistent pattern of reduction of GL-3

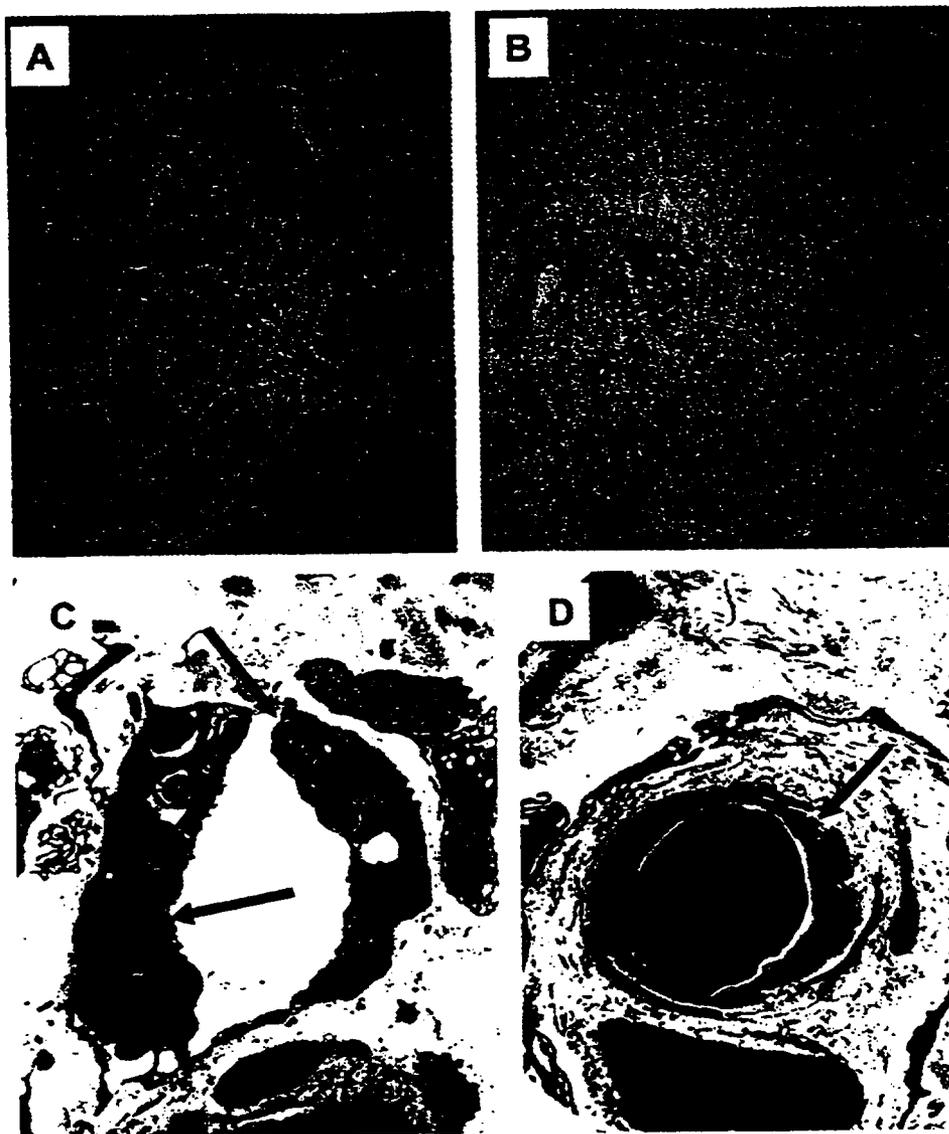


Figure 4 A and B, Immunohistochemistry for GL-3 in skin with verotoxin subunit B. Comparison of pretreatment (A) and posttreatment (B) photomicrographs shows that r-hGalA treatment resulted in marked clearance of GL-3 inclusions, particularly from the superficial vasculature of the papillary dermis. C and D, Electron micrographs of single superficial capillaries of the skin. Before treatment (C), the capillary endothelium was heavily laden with lamellar glycosphingolipid inclusions (arrows). After treatment (D), the endothelium of superficial capillaries was cleared of GL-3 inclusions (arrow). See text for details.

inclusions in the three vascular beds. As shown in figure 3C, GL-3 accumulation in the endothelium of interstitial capillaries declined in four of five patients (e.g., fig. 6A–6D) but increased in one. In two patients with paired glomerular capillary scores, endothelial GL-3 scores declined from 2 to 0 and from 1 to 0, respectively. For the arterioles, four paired samples showed declines in endothelial GL-3 content of ≥ 1 point (mean scores of 2.25 at baseline to 1.00 after treatment).

Response in other tissue types varied considerably.

Prominent reductions occurred following treatment in glomerular mesangial cells (from 2.00 at baseline to 0.00 in group C; $n = 2$) and in cortical interstitial cells (2.60 at baseline to 1.00 in groups C, D, and E; $n = 5$). The distal convoluted tubules and collecting ducts that, along with the glomerular podocytes, had the most prominent GL-3 storage in the kidney at baseline were difficult to evaluate but appeared to trend to reduced scores. The glycosphingolipid deposits in the glomerular podocytes appeared unresponsive to treatment. Electron-micro-

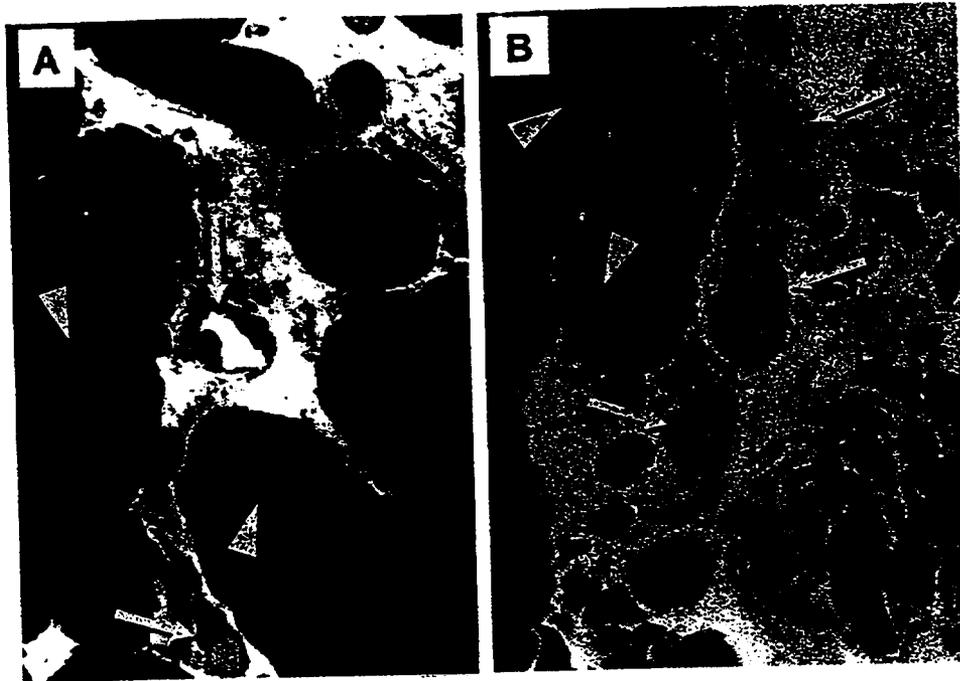


Figure 5 Photomicrographs of interstitial capillaries of the myocardium before (A) and after (B) treatment (methylene blue/azure II stain). Pretreatment (A), small-to-medium-sized glycosphingolipid inclusions were seen in the endothelium of small interstitial capillaries (arrows; score 2). After treatment (B), glycosphingolipid inclusions were cleared from the endothelium of small capillaries (arrows). The GL-3 inclusions in the cardiomyocytes (arrowheads) were less responsive to treatment. See text for details.

scopic evaluations, performed on a small number of samples, supported the findings from light microscopy.

Clinical Findings

Pre- and posttreatment EKGs, echocardiograms, and renal MRIs were unchanged. Patients anecdotally reported an increased ability to sweat and less fatigue. Pain was assessed by the Short Form McGill Pain Questionnaire. Compared to the pretreatment baseline results, the “overall pain” and “present pain intensity” scores were significantly improved after five infusions at all doses ($P = .03$ and $P = .004$, respectively). In addition, the SF-36 quality-of-life questionnaire indicated improvement posttreatment for three indices: bodily pain, general health, and vitality.

Safety Evaluation

Of the 15 patients, 13 completed all five r-h α GalA infusions, which were generally well tolerated. The most common adverse event was a transient mildly-to-moderately increased blood pressure during infusions, which did not require treatment and returned to normal during or immediately after infusion. There were no significant changes in blood indices or blood and urine chemistries. Patient 14 (group E), who had discontinued a 7-mo anticoagulant treatment for a deep vein thrombosis of a

lower extremity prior to joining the study, complained of mild chest pain the day after he received his last r-h α GalA infusion. He was diagnosed with a pulmonary embolus, was retreated with anticoagulant therapy, and recovered without complication.

Of 15 patients, 8 developed IgG antibodies specific to r-h α GalA. Of these, the four who experienced symptoms compatible with hypersensitivity-type reactions had seroconverted. Two of these patients (patient 5, group B, and patient 7, group C), had symptoms suggestive of allergic reactions during infusion 4 and did not tolerate reinfusion, whereas the other two patients were successfully retreated. Importantly, despite this antibody response, mean pharmacokinetic values did not change between the first and fifth infusions for all patients studied (fig. 7).

Discussion

The primary finding of this phase 1/2 trial was that infused r-h α GalA safely and effectively cleared the accumulated GL-3 from endothelium of the liver, skin, heart, and kidney—major sites of pathology in classical Fabry disease. The demonstration that lysosomal glycosphingolipid accumulation in the microvasculature and other cell types was reversible provided “proof of principle” for enzyme-replacement therapy in this disease. Since

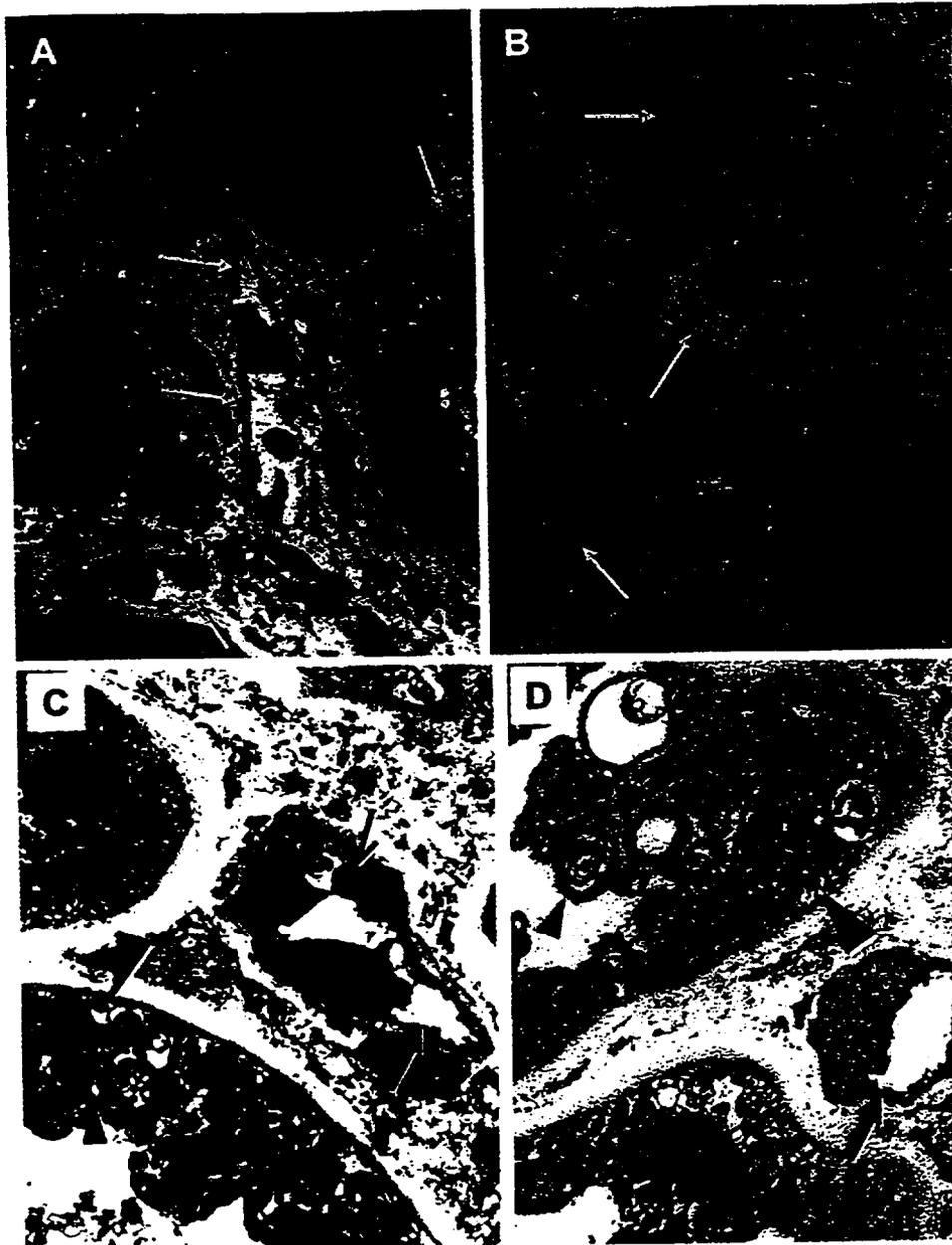


Figure 6 Light and electron micrographs of kidney pre- and posttreatment (methylene blue/azure II stain). *A* and *B*, Light microscopy of capillaries in the intertubular interstitium. Before treatment (*A*), the capillary endothelium (*arrows*) is heavily laden with glycosphingolipid inclusions, some impinging on the lumen (score 3). After treatment (*B*), most of the capillaries (*arrows*) have been cleared of glycosphingolipid inclusions (score 0). *C* and *D*, Electron micrographs of kidney cortex with examples of an intertubular capillary (*arrow*) and neighboring distal convoluted tubule (*arrowhead*). Before treatment (*C*), numerous electron-dense glycosphingolipid inclusions are seen in the capillary endothelium (*small arrow*). In the adjacent distal convoluted tubule, the epithelium is heavily packed with numerous electron-dense lamellar lysosomal inclusions (*small arrowhead*). After treatment (*D*), the endothelium of a small capillary (*arrow*) has no evidence of glycosphingolipid inclusions. In the adjacent distal convoluted tubule (*arrowhead*), the number and distribution of lysosomal inclusions appear similar; however, the lamellar architecture of the individual lysosomes appears less tightly organized (*small arrowhead*), suggesting enzymatic hydrolysis of the accumulated GL-3.

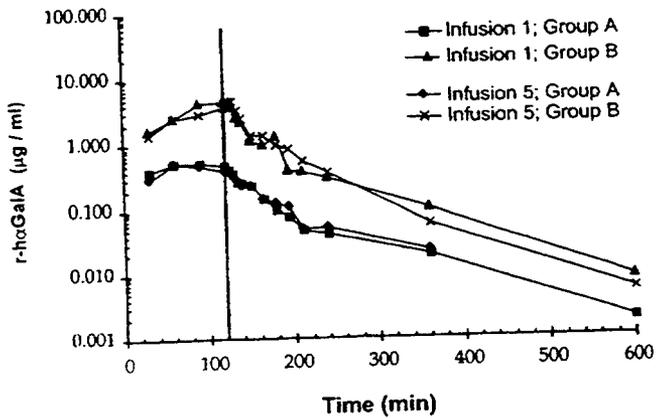


Figure 7 Comparison of the pharmacokinetics of r-h α GalA for infusions 1 and 5. Comparison of mean concentration-time data for groups A and B. R-h α GalA was infused over 120 min. Infusion 1: group A (\bullet), group B (\blacktriangle); infusion 5: group A (\blacklozenge), group B (\times). See text for details.

“cardiac variants” with Fabry disease lack microvascular glycosphingolipid accumulation and the major clinical manifestations of classically affected patients (Elder et al. 1990; von Scheidt et al. 1991; Desnick et al. 2001), it is anticipated that the clearance of GL-3 from the microvasculature and other tissue sites of pathology should lead to significant physiologic and clinical improvement.

Beyond the microvascular GL-3 clearance, the glycosphingolipid tissue load and response to r-h α GalA treatment varied. Presumably, the different cellular glycosphingolipid levels reflected endogenous glycosphingolipid synthesis and cell turnover rates, whereas the differential clearance was a function of cellular and lysosomal enzyme uptake and stability. In the liver, which had the highest uptake of enzyme on the basis of the preclinical studies in “Fabry mice” (Ioannou et al. 2001), all dose regimens markedly reduced GL-3 levels; there was an 84% mean clearance of hepatic GL-3 by ELISA in patients with pre- and posttreatment biopsies (table 2). Morphologic examination revealed that the two principal hepatic reservoirs of glycosphingolipid—the endothelial cells of the sinusoids and the Kupffer cells—were almost totally cleared of glycosphingolipid inclusions. In comparison to other tissues studied, baseline endomyocardial GL-3 levels were the highest of those in all tissues studied, an order of magnitude greater than those in kidney (table 2). Histologic analysis revealed that the majority of GL-3 accumulation was in the cardiomyocytes and that the storage was not remarkably changed after five doses of r-h α GalA at 1 or 3 mg/kg. Similar results were documented by the ELISA determinations (table 2). Longer-term enzyme replacement presumably is required to reduce the sub-

stantial GL-3 accumulation in cardiomyocytes. Of note, preclinical studies in the “Fabry mouse” indicated that r-h α GalA activity was detectable in the heart when 3.0 mg/kg was infused (Ioannou et al. 2001).

Although the number of paired pre- and posttreatment renal biopsies for histologic analysis was limited, all three vascular beds showed the same response to treatment, a prominent reduction in GL-3 content from the endothelium. Total renal GL-3 levels, determined by ELISA, in four of the five patients with pre- and post-biopsy data also revealed a mean 82% reduction after treatment. Because the total vascular GL-3 content is a relatively small component of the total renal GL-3 accumulation, and because the podocyte inclusions were unchanged, most of the GL-3 reduction must have been from the tubules, the only other major storage site. Taken together with the reduction in vascular endothelial GL-3 levels, these results suggest potential benefit for kidney function.

In general, the biochemical and histologic assessments indicated that GL-3 clearance was dose and tissue-dependent, analogous to the findings of r-h α GalA replacement in “Fabry mice” (Ioannou et al. 2001). The biweekly dose regimens proved to be more effective than the every 48 h regimens, the latter included to assess the effects of a rapid, high-dose schedule (i.e., a “loading dose”), similar to that used in the preclinical studies (Ioannou et al. 2001). It was reasoned that administration every 48 h would achieve the highest enzyme concentration in lysosomes and the greatest plasma and tissue GL-3 clearance, particularly since the tissue half-life of the enzyme in the “Fabry mouse” was 2–4 d. However, the biweekly regimens unexpectedly cleared more substrate, the 1- and 3-mg/kg doses being more effective than the 0.3-mg/kg dose. Although assessment of GL-3 clearance in heart and kidney was limited, because of the small number of optional paired biopsies from patients in groups C–E, the greatest clearance was observed in patients in group C, who received 3 mg/kg biweekly. In addition, there was a direct effect of dose on plasma GL-3 levels. For example, plasma GL-3 was cleared from the circulation after the first infusion in patients in the 1.0- and 3.0-mg/kg biweekly dosing groups (fig. 2), whereas plasma GL-3 levels in the 0.3-mg/kg dosing group decreased with each infusion, reaching their lowest levels at infusion 4 or 5. Of interest, GL-3 reaccumulation in the plasma and tissues of “Fabry mice” following a single r-h α GalA dose (3 mg/kg) suggest that the plasma GL-3 level might reflect the total body load of substrate (Ioannou et al. 2001). Analogously, the plasma GL-3 concentration may provide a noninvasive indicator of tissue GL-3 clearance in patients with Fabry disease.

Clinically, the patients reported decreased pain and an increased ability to sweat, both findings consistent

with GL-3 clearance from microvascular endothelial cells. Improvements in several quality-of-life measures also were noted. However, assessments of pain and quality of life require more rigorous evaluation in a larger, double-blind study, to minimize possible placebo effects.

This phase 1/2 clinical trial also evaluated the safety of r-h α GalA infusions. Although the enzyme infusions were generally well tolerated, it was expected that most patients would raise antibodies to r-h α GalA, since classically affected males with Fabry disease are cross-reactive immunological material-negative for the α -Gal A protein (Desnick et al. 2001). In fact, four patients had mild-to-moderate hypersensitivity-type reactions: two with transient fever and chills, one with hives, and one with tachycardia. Although they responded to symptomatic treatment, two of these four patients were unable to complete their fifth infusions. Immunologic studies revealed that six (67%) of the nine patients on the biweekly dosing schedule seroconverted after the second or third infusion, whereas two (33%) of six patients on the every-48-h regimens seroconverted after the fifth infusion (day 9). In all cases, seroconversion was an IgG response, and almost identical pharmacokinetic profiles at the first and last infusions for both biweekly (fig. 7) and every-48-h (data not shown) infusion schedules indicated that antibodies were not neutralizing. Also, urine chemistry, blood cytology, and tissue-histology results were not altered in response to adverse reactions associated with enzyme infusions. On the basis of these findings and of previous experience with management of enzyme infusions for Gaucher disease (Grabowski et al. 1998; Mistry 1999), pretreatment with antipyretics and antihistamines, as well as decreasing the rate of infusion, are recommended for subsequent studies.

The most common adverse event associated with enzyme administration was a mild, transient increase in blood pressure, which did not require medical intervention or prevent patients who entered the trial with hypertension from safely completing their infusions. Only two serious adverse events occurred, and of these only one, an allergic reaction, was attributable to r-h α GalA administration. The other serious adverse event was a pulmonary embolism in a patient who recently discontinued anticoagulation treatment for a previous deep vein thrombosis. Of the other recorded adverse events, none was attributed to enzyme administration; however, several were associated with biopsy procedures.

In summary, the phase 1/2 clinical trial of r-h α GalA replacement in classically affected patients with Fabry disease demonstrated that the infused enzyme generally was well tolerated and that the expected infusion reactions following seroconversion were mild and con-

servatively managed. This trial provided the "proof of principle" that enzyme replacement could reverse the GL-3 accumulation in key sites of pathology. These results also indicated that the clearance of GL-3 from tissue lysosomes was dose-dependent and that enzyme could reach and hydrolyze the accumulated GL-3 in lysosomes. Finally, these studies provide the basis for a phase 3 pivotal trial with a greater number of patients to further evaluate the safety and efficacy of r-h α GalA replacement in Fabry disease.

Acknowledgments

The authors express their appreciation to the patients who participated in this trial, to our study coordinators (Melissa Nunn, B.A., and Athena Palearis, R.N.); to our medical fellows (Patricia Ashton-Prolla, M.D., and Kamal Topaloglu, M.D.); to our physician consultants (Mark W. Babyatsky, M.D., Meir Shinnar, M.D., and Swan N. Thung, M.D.); to the nursing and support staff of the General Clinical Research Center at the Mount Sinai School of Medicine, New York; and to our collaborators at the Genzyme Corporation (Richard Moscicki, M.D.; Susan Richards, Ph.D.; P. K. Tandon, Ph.D.; and their respective staffs). This work was supported in part by grants from the National Institutes of Health, including a Merit Award (5 R37 DK34045), a grant (5 M01 RR00071) for the Mount Sinai General Clinical Research Center, a grant (5 P30 HD28822) for the Mount Sinai Child Health Research Center, and a research grant from the Genzyme Corporation.

Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for Fabry disease [MIM 301500])

References

- Bishop DF, Calhoun DH, Bernstein HS, Hantzopoulos P, Quinn M, Desnick RJ (1986) Human α -galactosidase A: nucleotide sequence of a cDNA clone encoding the mature enzyme. *Proc Natl Acad Sci USA* 83:4859-4863
- Brady RO, Tallman JF, Johnson WG, Gal AE, Leahy WR, Quirk JM, Dekaban AS (1973) Replacement therapy for inherited enzyme deficiency: use of purified ceramidetrihexosidase in Fabry's disease. *N Engl J Med* 289:9-14
- Colombi A, Kostyal A, Bracher R, Gloor F, Mazzi R, Tholen H (1967) Angiokeratoma corporis diffusum: Fabry's disease. *Helv Med Acta* 34:67-83
- Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W (1973) Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes: α -galactosidase activities in plasma, serum, urine, and leukocytes. *J Lab Clin Med* 81: 157-171
- Desnick RJ, Dean KJ, Grabowski G, Bishop DF, Sweeley CC (1979) Enzyme therapy in Fabry disease: differential in vivo

- plasma clearance and metabolic effectiveness of plasma and splenic α -galactosidase A isozymes. *Proc Natl Acad Sci USA* 76:5326-5330
- (1980) Enzyme therapy XVII: metabolic and immunologic evaluation of α -galactosidase A replacement in Fabry disease. *Birth Defects Orig Artic Ser* 16:393-413
- Desnick RJ, Ioannou YA, Eng CM (1995) α -galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) *The metabolic and molecular bases of inherited diseases*, 7th ed. McGraw-Hill, New York, pp 2741-2784
- (2001) α -galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Kinzler KE, Vogelstein B (eds) *The metabolic and molecular bases of inherited diseases*, 8th ed. McGraw-Hill, New York, pp 3733-3774
- Elleder M, Bradova V, Smid F, Budesinsky M, Harzer K, Kustermann-Kuhn B, Ledvinova J, Belohlavek, Kral V, Dorazilova V (1990) Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease: report on a case simulating hypertrophic non-obstructive cardiomyopathy. *Virchows Arch A Pathol Anat Histopathol* 417:449-455
- Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ (1993) Nature and frequency of mutations in the α -galactosidase A gene that cause Fabry disease. *Am J Hum Genet* 53:1186-1197
- Grabowski GA, Leslie N, Wenstrup R (1998) Enzyme therapy for Gaucher disease: the first 5 years. *Blood Rev* 12:115-133
- Ioannou YA, Bishop DF, Desnick RJ (1992) Overexpression of human α -galactosidase A results in its intracellular aggregation, crystallization in lysosomes and selective secretion. *J Cell Biol* 119:1137-1150
- Ioannou YA, Zeidner KM, Gordon RE, Desnick RJ (2001) Fabry disease: preclinical studies demonstrate the effectiveness of α -galactosidase A replacement in enzyme-deficient mice. *Am J Hum Genet* 68:14-25
- Mapes CA, Anderson RL, Sweeley CC, Desnick RJ, Krivit W (1970) Enzyme replacement in Fabry's disease, an inborn error of metabolism. *Science* 169:987-989
- Matsuura F, Ohta M, Ioannou YA, Desnick RJ (1998) Human α -galactosidase A: characterization of the N-linked oligosaccharides on the intracellular and secreted glycoforms overexpressed by Chinese hamster ovary cells. *Glycobiology* 8:329-339
- Melzack R (1987) The short-form McGill pain questionnaire. *Pain* 30:191-197
- Mistry PK (1999) Gaucher's disease: a model for modern management of a genetic disease. *J Hepatol* 30:1-5
- Schiffmann R, Murray GJ, Treco D, Daniel P, Sellos-Moura M, Myers M, Quirk JM, Zirzow GC, Borowski M, Loveday K, Anderson T, Gillespie F, Oliver KL, Jeffries NO, Doo E, Liang TJ, Kreps C, Gunter K, Frei K, Crutchfield K, Selden RF, Brady RO (2000) Infusion of α -galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. *Proc Natl Acad Sci USA* 97:365-370
- von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, Olsen EG, Christomanou H, Kandolf R, Bishop DF, Desnick RJ (1991) An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med* 324:395-399
- Wang AM, Ioannou YA, Zeidner KM, Gotleb RW, Dikman S, Stewart CL, Desnick RJ (1996) Generation of a mouse model with α -galactosidase A deficiency. *Am J Hum Genet Suppl* 59:A208
- Ware J, Snow K, Kosinski M, Gandek B (1997) SF-36 health survey manual and interpretation guide. The Health Institute, New England Medical Center, Boston
- Zeidner K, Desnick R, Ioannou Y (1999) Quantitative determination of globotriaosylceramide by immunodetection of glycolipid-bound recombinant verotoxin B subunit. *Anal Biochem* 267:104-113

SAFETY AND EFFICACY OF RECOMBINANT HUMAN α -GALACTOSIDASE A REPLACEMENT THERAPY IN FABRY'S DISEASE

CHRISTINE M. ENG, M.D., NATHALIE GUFFON, M.D., WILLIAM R. WILCOX, M.D., PH.D.,
DOMINIQUE P. GERMAIN, M.D., PH.D., PHILIP LEE, M.R.C.P., D.M., PH.D., STEVE WALDEK, M.B., B.CH.,
LOUIS CAPLAN, M.D., GABOR E. LINTHORST, M.D., AND ROBERT J. DESNICK, PH.D., M.D.,
FOR THE INTERNATIONAL COLLABORATIVE FABRY DISEASE STUDY GROUP

ABSTRACT

Background Fabry's disease, lysosomal α -galactosidase A deficiency, results from the progressive accumulation of globotriaosylceramide and related glycosphingolipids. Affected patients have microvascular disease of the kidneys, heart, and brain.

Methods We evaluated the safety and effectiveness of recombinant α -galactosidase A in a multicenter, randomized, placebo-controlled, double-blind study of 58 patients who were treated every 2 weeks for 20 weeks. Thereafter, all patients received recombinant α -galactosidase A in an open-label extension study. The primary efficacy end point was the percentage of patients in whom renal microvascular endothelial deposits of globotriaosylceramide were cleared (reduced to normal or near-normal levels). We also evaluated the histologic clearance of microvascular endothelial deposits of globotriaosylceramide in the endomyocardium and skin, as well as changes in the level of pain and the quality of life.

Results In the double-blind study, 20 of the 29 patients in the recombinant α -galactosidase A group (69 percent) had no microvascular endothelial deposits of globotriaosylceramide after 20 weeks, as compared with none of the 29 patients in the placebo group ($P < 0.001$). Patients in the recombinant α -galactosidase A group also had decreased microvascular endothelial deposits of globotriaosylceramide in the skin ($P < 0.001$) and heart ($P < 0.001$). Plasma levels of globotriaosylceramide were directly correlated with clearance of the microvascular deposits. After six months of open-label therapy, all patients in the former placebo group and 98 percent of patients in the former recombinant α -galactosidase A group who had biopsies had clearance of microvascular endothelial deposits of globotriaosylceramide. Mild-to-moderate infusion reactions (i.e., rigors and fever) were more common in the recombinant α -galactosidase A group than in the placebo group.

Conclusions Recombinant α -galactosidase A replacement therapy cleared microvascular endothelial deposits of globotriaosylceramide from the kidneys, heart, and skin in patients with Fabry's disease, reversing the pathogenesis of the chief clinical manifestations of this disease. (N Engl J Med 2001;345:9-16.)

Copyright © 2001 Massachusetts Medical Society.

FABRY'S disease is an X-linked inborn error of glycosphingolipid catabolism due to deficient lysosomal α -galactosidase A activity.¹ In patients with the classic form of the disease, progressive accumulation of globotriaosylceramide and related glycosphingolipids in vascular endothelial lysosomes of the kidneys, heart, skin, and brain leads to the main disease manifestations. The clinical onset is in childhood and is characterized by severe acroparesthesias, angiokeratoma, corneal and lenticular opacities, and hypohidrosis. Over time, microvascular disease of the kidneys, heart, and brain progresses, leading to early death.¹ Treatment is limited to symptomatic management of pain and the end-stage complications of renal failure, cardiac disease, and strokes.

Early trials demonstrated the feasibility of enzyme replacement to correct the metabolic defect in Fabry's disease.²⁻⁴ A phase 1 trial demonstrated reductions of globotriaosylceramide in the liver and in urinary sediment with a single dose of recombinant α -galactosidase A.⁵ A phase 1 and 2 open-label dose-escalation study of replacement therapy with recombinant α -galactosidase A in 15 male patients with classic Fabry's disease demonstrated that repeated administration (a total of five infusions) was safe and effective in clearing plasma globotriaosylceramide and microvascular endothelial deposits of globotriaosylceramide from target tissues.⁶ Plasma and tissue clearance of globotriaosylceramide was observed for all dose regimens, and the effect was most pronounced at higher doses. Therefore, we evaluated the safety and efficacy of recombinant α -galactosidase A replacement therapy in Fabry's disease in a multicenter, randomized, double-blind, placebo-controlled trial and subsequent open-label study.

METHODS**Patients**

Eligible patients had an enzymatically confirmed diagnosis of classic Fabry's disease, had a level of activity of α -galactosidase A of less

From the Mount Sinai School of Medicine, New York (C.M.E., R.J.D.); Hôpital Edouard Herriot, Lyons, France (N.G.); Cedars-Sinai Burns and Allen Research Institute, UCLA School of Medicine, Los Angeles (W.R.W.); Hôpital Européen Georges Pompidou, Paris (D.P.G.); University College London Hospitals, London (P.L.); Hope Hospital, Salford, Manchester, United Kingdom (S.W.); Beth Israel Deaconess Medical Center, Boston (L.C.); and Academisch Medisch Centrum, Amsterdam (G.E.L.). Address reprint requests to Dr. Desnick at the Department of Human Genetics, Box 1498, Mount Sinai School of Medicine, Fifth Ave. at 100th St., New York, NY 10029, or at rjd.fabry@mssm.edu.

than 1.5 nmol per hour per milliliter in plasma or less than 4 nmol per hour per milligram in leukocytes,⁷ and were at least 16 years old. Patients were excluded if their serum creatinine concentration exceeded 2.2 mg per deciliter (194.5 μ mol per liter), if they were undergoing dialysis, or if they had undergone kidney transplantation.

Clinical and Biochemical Assessments

Evaluations including a medical history taking, routine chemical analyses, and hematologic indexes were obtained and a physical examination was performed at base line and before each infusion. Echocardiograms were obtained and plasma and 24-hour urinary sediments were collected at base line, after week 20 of the double-blind study, and after six months of open-label treatment. Glomerular filtration rates were measured in terms of inulin clearance at base line and after six months of the extension study. Concentrations of globotriaosylceramide in plasma, tissue, and urinary sediment⁸ were determined by a quantitative enzyme-linked immunosorbent assay (ELISA).⁹ Before each infusion, the presence or absence of antibody against recombinant α -galactosidase A was assessed by ELISA, and the results were confirmed by a radioimmunoprecipitation assay.¹⁰

Study Protocol

Enrollment in the double-blind study began on March 22, 1999, and ended on December 3, 1999. The open-label study began on October 26, 1999. Patients were randomly assigned to receive recombinant α -galactosidase A (agalsidase beta; Fabrazyme, Genzyme, Cambridge, Mass.) at a dose of 1 mg per kilogram of body weight or placebo (phosphate-buffered mannitol). Both agents were administered intravenously at a rate of 0.25 mg per minute every other week for 20 weeks (for a total of 11 infusions). Before each infusion patients were pretreated with 1000 mg of acetaminophen and 25 to 50 mg of hydroxyzine. Ibuprofen, prednisone, or both were also used in a few patients for infusion-related reactions. After the double-blind trial, all patients received recombinant α -galactosidase A in an open-label fashion at a dose of 1 mg per kilogram every other week, but the infusion rates were increased as tolerated, reducing the length of the infusion. The institutional review boards at all sites approved the double-blind and open-label protocols, and all patients gave written informed consent.

Tissue Assessments

Kidney specimens were obtained by ultrasound-guided biopsy, heart specimens were obtained through an endomyocardial catheter with the use of a biptome, and 3-mm skin specimens were obtained by punch biopsy at base line, after infusion 11 (week 20), and after six months of the open-label study. Tissue sections (1 μ m) were stained with methylene blue-azure II. Each of the three types of biopsy specimen was assessed for microvascular endothelial deposits of globotriaosylceramide by a different group of three pathologists. None of the nine pathologists were aware of the patients' treatment assignments or the times at which the specimens were obtained.

Specimens with no microvascular endothelial deposits of globotriaosylceramide or only trace amounts (normal or nearly normal) were given a score of 0; specimens in which the majority of vessels had evidence of a single endothelial inclusion were given a score of 1; specimens that contained multiple vessels with multiple sites of single or multiple inclusions were given a score of 2; and specimens that had large accumulations of inclusions with some clusters at the juxtannuclear region and around cytoplasmic borders and bulging of the vessel lumens were given a score of 3. Renal-biopsy specimens that were initially given a score of 0 or 1 were reevaluated by the three renal pathologists with the use of a slightly modified scoring system. In this system, specimens with no inclusions were given a score of 0; those with one small granule (approximately 0.2 μ m) were designated as having trace evidence; those with multiple discrete granules were given a score of 1; those with single or multiple aggregates of granules were given a score of 2; and those with aggregates of granules within the endothelium that caused the distortion of the luminal endothelial cell surface were given a score of 3.

Evaluation of Efficacy

An average of 233 capillaries in each renal-biopsy specimen were assessed by each renal pathologist. The primary efficacy end point of the double-blind study required more than 50 percent of the renal interstitial capillaries in each specimen to have a score of 0, less than 5 percent to have a score of 1 or greater, and the remainder to be designated as having trace evidence of microvascular endothelial deposits of globotriaosylceramide after week 20. For each biopsy specimen, a majority score was determined from the three pathologists' scores.

Secondary end points were also assessed at base line and after the week-20 infusion and consisted of the composite score for microvascular endothelial deposits of globotriaosylceramide in the heart, kidney, and skin specimens (scores were calculated per organ and summed for all organs) and the change from base line in the concentrations of globotriaosylceramide in urinary sediment and kidney specimens and the level of pain, as assessed by the short form of the McGill Pain Questionnaire.¹¹ Scores on this questionnaire can range from 0 to 45, with higher scores indicating severe pain intensity.

Statistical Analysis

We used chi-square tests to analyze the proportion of patients in the recombinant α -galactosidase A group and the placebo group with a renal-biopsy score of 0 after week 20 of the double-blind study and after six months of the open-label study. We used two-sample, two-tailed tests for all analyses. A P value of 0.05 or less was considered to indicate statistical significance. Changes in the concentrations of globotriaosylceramide in urinary sediment and kidney specimens on ELISA were ranked individually, and the rank-sum score for each patient was obtained. We used a two-sample Wilcoxon rank-sum test to assess the change from base line to the end of the double-blind study (after week 20). We used t-tests to compare the mean change in the level of pain from base line to the end of the double-blind study (after week 20) for each treatment group. The Genzyme Biostatistics group held the data and analyzed the data, with the help of consulting academic biostatisticians.

We used the 36-item Medical Outcomes Study Short-Form General Health Survey (SF-36)¹² to evaluate the patients' quality of life. This multi-item scale measures eight health-related aspects: physical function, social function, physical role, emotional role, mental health, energy, pain, and general health perception. Scores on each aspect can range from 0 (worst) to 100 (best). The results were evaluated according to established guidelines,¹² and we used a Wilcoxon signed-rank test to compare the mean change in scores from base line in each group. We used an analysis of variance to compare the differences between groups in the changes in the mean glomerular filtration rate from base line to six months of the open-label study.

RESULTS

Characteristics of the Patients

The base-line characteristics of the 58 patients assigned to the two treatment groups were similar (Table 1).

Double-Blind Study

Renal Capillary Endothelial Clearance of Globotriaosylceramide

The primary efficacy end point was the percentage of patients in each group who were free of microvascular endothelial deposits of globotriaosylceramide in renal-biopsy specimens (i.e., who had a score of 0) after 20 weeks of treatment (11 infusions) in the double-blind study. The end point was reached by 20 of the 29 patients in the recombinant α -galactosidase A

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	RECOMBINANT α -GALACTOSIDASE A GROUP (N=29)	PLACEBO GROUP (N=29)
Age (yr)		
Mean	32.0 \pm 9.4	28.4 \pm 11.4
Range	16-48	17-61
Weight (kg)	67.3 \pm 9.9	69.6 \pm 13.4
Height (cm)	175.7 \pm 8.3	175.6 \pm 8.3
Sex (no.)		
Male	27	29
Female	2	0
Race (no.)		
White	27	26
Nonwhite	2	3
Plasma globotriaosylceramide (ng/ml)	14.5 \pm 10.5	14.6 \pm 9.6
Glomerular filtration rate (ml/min)	83.0 \pm 22.0	96.6 \pm 35.3
Serum creatinine (mg/dl)†	0.8 \pm 0.2	0.8 \pm 0.2

*Plus-minus values are means \pm SD.

†To convert the values for creatinine to micromoles per liter, multiply by 88.4.

group (69 percent), as compared with none of the 29 patients in the placebo group ($P < 0.001$; odds ratio, 0.0). Eight of the remaining nine patients in the recombinant α -galactosidase A group had a score of 1 (the scores of six of these patients had improved, and the scores of two had not changed). The ninth patient had a missing biopsy specimen and so was assigned a score of 3. An analysis of sensitivity, in which a maximum of 1 percent of the capillaries could be given a score of 1 or greater, as opposed to the original requirement of less than or equal to 5 percent, did not change the outcome ($P < 0.005$). These results for the three renal pathologists were uniform.

Secondary End Points

The individual scores for the kidney-, heart-, and skin-biopsy specimens as well as the composite scores for all three types of specimens were compared at base line and after the week-20 infusion (Table 2). Although both groups had similar base-line scores for each type of specimen ($P = 0.53$), the patients in the recombinant α -galactosidase A group had significantly lower scores for each type of specimen after the week-20 infusion than did the patients in the placebo group ($P < 0.001$ for all three comparisons). In addition, the median percent changes in the kidney and urinary concentrations of globotriaosylceramide differed between the patients in the recombinant α -galactosidase A group and the patients in the placebo group (23.3 percent decrease vs. 42.8 percent increase and 34.1 percent decrease vs. 6.2 percent decrease, respectively). The rank-sum scores for kidney and urinary-sediment concentrations of globotriaosylceramide had

decreased significantly in the recombinant α -galactosidase A group, but not in the placebo group (median change, 32.5 percent decrease vs. 48.0 percent decrease; $P = 0.003$).

Although both groups had low scores on all five scales of the short form of the McGill Pain Questionnaire at base line, statistically significant decreases in the scores were observed at week 20 in both treatment groups (Fig. 1). There was no significant difference between groups after week 20 in any pain assessment ($P > 0.05$ for all comparisons), possibly because of a placebo effect.

Clearance of Globotriaosylceramide in Plasma

Figure 2 shows clearance of globotriaosylceramide from plasma by week 14 of treatment with recombinant α -galactosidase A; in contrast, the plasma concentrations in the placebo group did not change significantly during the double-blind study ($P < 0.001$ for the comparison between the groups). Plasma concentrations of globotriaosylceramide were undetectable (< 1.2 ng per microliter) after week 20 in all 20 patients who had no microvascular endothelial deposits of globotriaosylceramide in renal-biopsy specimens after week 20 of treatment. Five of eight patients in the recombinant α -galactosidase A group who had a renal score of 1 after week 20 had undetectable plasma concentrations of globotriaosylceramide after week 20, and three had concentrations ranging from 12 to 94 percent (mean, 35.3 percent) of their base line values. The patient who had been assigned a score of 3 because of a missing biopsy specimen at week 20 had a plasma globotriaosylceramide concentration of 3.9 ng per microliter.

Quality of Life

Patients in the recombinant α -galactosidase A group had significant improvements in two components of the SF-36 (physical role and emotional role), whereas patients in the placebo group had significant improvements in the physical role and body-pain components of the SF-36.

Open-Label Extension Study

All 58 patients enrolled in the open-label study. After six months of treatment with recombinant α -galactosidase A, 98 percent of patients in whom a biopsy was performed at this time (42 of 43) had a score of 0 on histologic analysis of microvascular endothelial deposits of globotriaosylceramide in kidney specimens, 96 percent (45 of 47) had such results for skin specimens, and 75 percent (24 of 32) had such results for heart specimens (Table 3). The results were similar when the analysis included only the patients who crossed over from placebo to recombinant α -galactosidase A: 100 percent, 96 percent, and 67 percent, respectively. In 95 percent of the patients who had had a biopsy during the open-label study and who received

TABLE 2. MEAN CHANGES IN INDIVIDUAL AND COMPOSITE SCORES FOR MICROVASCULAR ENDOTHELIAL DEPOSITS OF GLOBOTRIAOSYLCERAMIDE IN KIDNEY-, HEART-, AND SKIN-BIOPSY SPECIMENS FROM BASE LINE TO AFTER THE WEEK-20 INFUSION.*

SPECIMEN	No. OF PATIENTS	BASE LINE	WEEK 20	MEAN CHANGE	P VALUE†
			score		
Kidney					<0.001
Recombinant α -galactosidase A	29	1.9 \pm 0.8	0.4 \pm 0.7	-1.6 \pm 1.2	
Placebo	29	2.2 \pm 0.7	2.1 \pm 0.8	-0.1 \pm 1.1	
Heart					<0.001
Recombinant α -galactosidase A	29	0.9 \pm 0.4	0.3 \pm 0.5	-0.6 \pm 0.7	
Placebo	29	0.9 \pm 0.5	1.2 \pm 0.6	0.2 \pm 0.8	
Skin					<0.001
Recombinant α -galactosidase A	29	2.1 \pm 0.7	0.0 \pm 0.0	-2.1 \pm 0.7	
Placebo	29	2.3 \pm 0.8	2.2 \pm 0.7	-0.1 \pm 1.0	
Composite					<0.001
Recombinant α -galactosidase A	29	4.9 \pm 1.5	0.7 \pm 0.8	-4.2 \pm 1.8	
Placebo	29	5.4 \pm 1.4	5.5 \pm 1.6	0.1 \pm 2.0	

*Plus-minus values are means \pm SD.

†P values are for the mean changes and were calculated with use of a t-test.

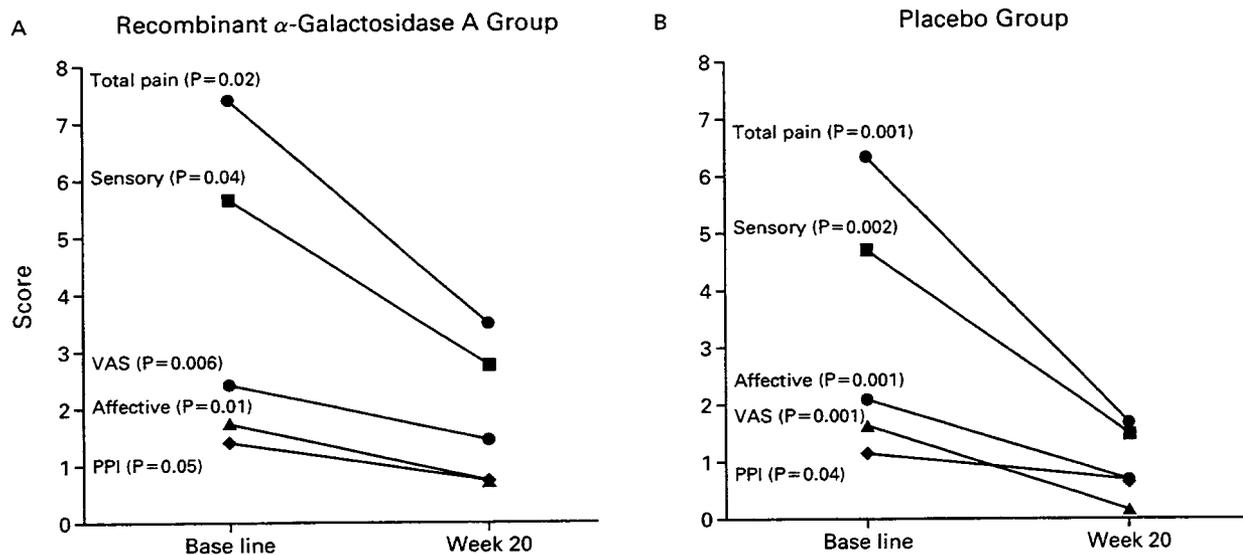


Figure 1. Change in Levels of Pain from Base Line to Week 20 of the Double-Blind Study in the Recombinant α -Galactosidase A Group (Panel A) and the Placebo Group (Panel B).

The short form of the McGill Pain Questionnaire was used to assess the level of sensory pain, affective pain, pain as measured on a visual analogue scale (VAS), and the present pain intensity (PPI). On this scale, higher scores indicate greater pain. The total pain score is the sum of the sensory and affective pain scores. There were significant reductions in all the mean pain measures within each treatment group, but no significant differences between the two groups.

recombinant α -galactosidase A during the double-blind study, the renal scores were maintained or further decreased after six months of open-label treatment. In addition, renal function, as measured by the glomerular filtration rate, did not change substantially from base line in either group after week 20 of the

double-blind study ($P=0.19$) or after six months of open-label treatment ($P=0.81$).

Safety

No significant changes from base line in the echocardiograms, electrocardiograms, or other safety as-

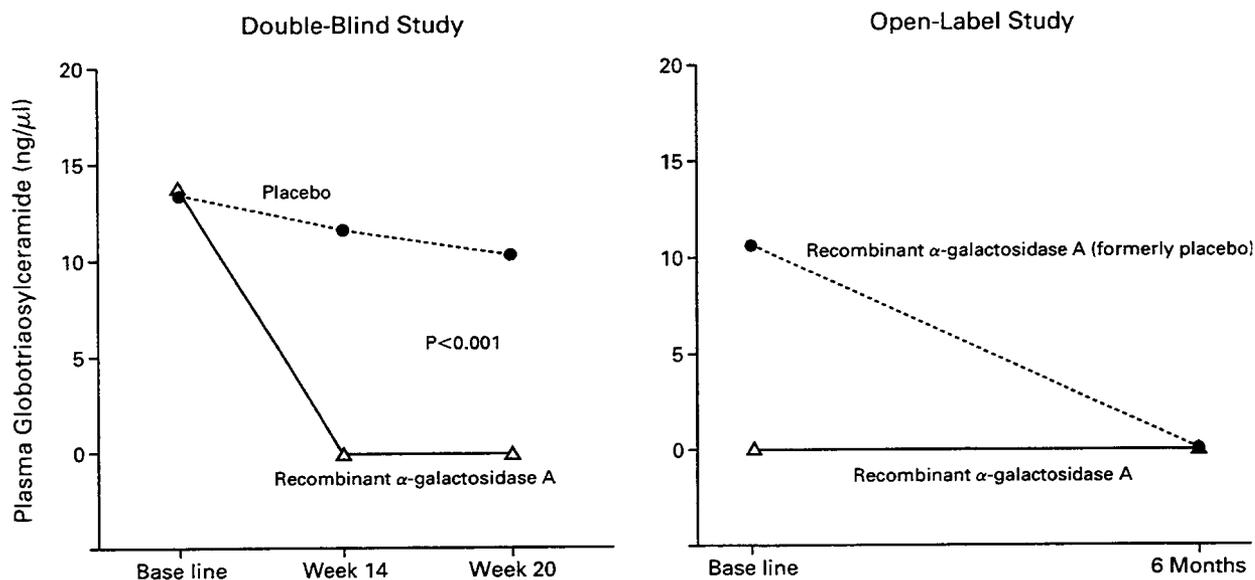


Figure 2. Median Plasma Concentrations of Globotriaosylceramide in the Recombinant α -Galactosidase A Group and the Placebo Group in the Double-Blind Study and the Open-Label Study.

Plasma levels of globotriaosylceramide were determined by a quantitative enzyme-linked immunosorbent assay at base line and week 20 of the double-blind study and after six months of open-label treatment. Plasma globotriaosylceramide values that were below the limit of detection (<1.2 ng per microliter) were recorded as 0. All patients who had been in the placebo group during the double-blind study received recombinant α -galactosidase A during the open-label study.

assessments in either group were observed after week 20 of the double-blind study or after six months of the open-label study. The infusions were generally well tolerated. Rigors and fever were the only treatment-related adverse events that occurred significantly more frequently in the recombinant α -galactosidase A group than in the placebo group during the double-blind study ($P=0.004$) (Table 4). Although not considered to be related to recombinant α -galactosidase A therapy, skeletal pain was the only other adverse event that occurred more frequently among enzyme-treated patients during the double-blind study ($P=0.02$). Transient mild-to-moderate infusion-associated reactions occurred in 59 percent of patients (34 of 58) during double-blind or open-label treatment. Reducing the infusion rate, administering preventive medications, or both measures controlled these reactions. A single patient had a positive skin test to recombinant α -galactosidase A after his eighth infusion during the open-label study, and treatment was discontinued.

IgG seroconversion occurred in 51 of the 58 patients who received recombinant α -galactosidase A (88 percent) during the study. Seroconversion did not affect the primary or secondary efficacy end points. For example, the distribution of the scores for renal specimens (0 vs. not 0) did not differ significantly between patients who did seroconvert and those who did not. In addition, 8 of 29 patients in the original recombinant α -galactosidase A group who had renal scores of

TABLE 3. NUMBER OF PATIENTS WITH A SCORE OF 0 ON HISTOLOGIC ANALYSIS OF MICROVASCULAR CAPILLARY ENDOTHELIAL DEPOSITS OF GLOBOTRIAOSYLCERAMIDE IN BIOPSY SPECIMENS AFTER 20 WEEKS OF DOUBLE-BLIND TREATMENT AND 6 MONTHS OF OPEN-LABEL TREATMENT WITH RECOMBINANT α -GALACTOSIDASE A.

SPECIMEN AND PREVIOUS DOUBLE-BLIND TREATMENT	No. EVALUATED AT 12 Mo	No. WITH SCORES OF 0 (%)	MONTHS OF RECOMBINANT α -GALACTOSIDASE A TREATMENT
Kidney			
Placebo	22	22 (100)	6
Recombinant α -galactosidase A	21	20 (95)	12
Total	43	42 (98)	—
Heart			
Placebo	15	10 (67)	6
Recombinant α -galactosidase A	17	14 (82)	12
Total	32	24 (75)	—
Skin			
Placebo	23	22 (96)	6
Recombinant α -galactosidase A	24	23 (96)	12
Total	47	45 (96)	—

TABLE 4. ADVERSE EVENTS THAT OCCURRED IN AT LEAST 10 PERCENT OF PATIENTS IN THE RECOMBINANT α -GALACTOSIDASE A GROUP DURING THE DOUBLE-BLIND STUDY.

ADVERSE EVENT	RECOMBINANT α -GALACTOSIDASE A GROUP (N=29)	PLACEBO GROUP (N=29)
	no. (%)	
Rigors	14 (48)*	0
Fever	7 (24)†	1 (3)
Headache	5 (17)	2 (7)
Chills	4 (14)	0
Pain related to Fabry's disease	3 (10)	1 (3)
Hypertension	3 (10)	0

*P=0.004 by Fisher's exact test.

†P=0.024 by Fisher's exact test.

1 after week 20 had a reduction in their scores to 0 during the open-label study. IgG titers had decreased in 15 of 26 patients in the recombinant α -galactosidase A group (58 percent) who seroconverted during the double-blind study when the titers were assessed after 12 months of treatment. In addition, one IgG-positive patient with a low titer became seronegative during this period. These observations serve to reduce concern about potential reactions associated with seroconversion.

DISCUSSION

During the past decade the safety and effectiveness of enzyme-replacement therapy have been demonstrated in patients with type 1 Gaucher's disease.^{13,14} In patients with this lysosomal storage disease, the infusion of human placental or recombinant acid β -glucosidase metabolized the accumulated substrate, reversed the disease-related abnormalities, and markedly improved the quality of life.^{13,14} We report the results of a randomized, double-blind, placebo-controlled trial and the first six months of an open-label extension study that demonstrate the safety and effectiveness of enzyme replacement in a second lysosomal disorder, Fabry's disease.

In patients with classic Fabry's disease, the chief debilitating manifestations result primarily from the progressive accumulation of microvascular endothelial deposits of globotriaosylceramide, leading to ischemia and infarction, particularly in the kidneys, heart, and brain.¹ In contrast, patients with the cardiac variant of the disease have residual α -galactosidase A activity (<10 percent of normal levels) and do not have vascular endothelial accumulation of glycosphingolipid.^{1,15,16} In these patients, left ventricular hypertrophy and mild proteinuria typically develop late in life, the life span

is normal, and the classic manifestations of the disease, including angiokeratoma, acroparesthesias, hypohidrosis, and renal failure, are absent.¹ Thus, the reversal of the underlying vascular endothelial abnormalities in patients with classic Fabry's disease should be therapeutic.

We found that 11 infusions of recombinant α -galactosidase A at a dose of 1 mg per kilogram over a 20-week period safely and effectively cleared the abnormalities in the capillary endothelium of the kidneys, heart, and skin of patients with classic Fabry's disease. The primary efficacy end point of our study directly addressed a fundamental cause of the most common and devastating feature of classic Fabry's disease: renal failure. After 20 weeks of treatment, complete or almost complete clearance of the accumulated renal microvascular endothelial deposits of globotriaosylceramide was achieved in 69 percent of the patients in the recombinant α -galactosidase A group, as compared with none of the patients in the placebo group (P<0.001). In addition, the concentration of globotriaosylceramide was significantly reduced in the urinary sediment of patients in the recombinant α -galactosidase A group, providing indirect evidence of the clearance of glycosphingolipids in renal tubules. Similar results were achieved with respect to the clearance of microvascular endothelial deposits of globotriaosylceramide from the heart (P<0.001) and skin (P<0.001).

The open-label extension study confirmed the results of the double-blind study and demonstrated that clearance was maintained or that microvascular endothelial deposits of globotriaosylceramide were further reduced in all three types of specimens assessed in patients who were treated with recombinant α -galactosidase A for about one year. Notably, the percentage of patients with clearance of the microvascular endothelial deposits of globotriaosylceramide in the endomyocardium increased from 67 percent after 20 weeks to 82 percent after 6 months of open-label treatment, indicating that the clearance of globotriaosylceramide may be tissue specific, depending on the dose and duration of treatment, the level of enzyme uptake, and the degree of substrate accumulation. Taken together, the results of the double-blind and open-label studies confirm that recombinant α -galactosidase A replacement therapy cleared the accumulated microvascular endothelial deposits of globotriaosylceramide and reversed the chief underlying abnormality in Fabry's disease. On the basis of the results of the preclinical,¹⁷ phase 1 and 2 dose-escalation,⁶ and double-blind studies, the plasma globotriaosylceramide level may be correlated with the accumulation of this glycosphingolipid in tissue and may provide a noninvasive indicator of systemic substrate clearance, analogous to serum glucose levels in patients with diabetes.

Most patients with the classic form of the disease have episodic acroparesthesias that are debilitating and markedly impair their quality of life. Patients in a phase

1 and 2 open-label study reported decreased severity of pain related to Fabry's disease.⁶ In our double-blind study, the severity of pain and the quality of life, as assessed by standardized instruments, were significantly improved in both groups, making it impossible to differentiate treatment-related effects from a placebo effect. For ethical reasons, patients who had been dependent on prophylactic drugs, analgesics, or both for years continued to take such medications during the study, a factor that may have minimized baseline scores and subsequent differences between groups. Studies are needed to determine the long-term effects of treatment with recombinant α -galactosidase A, perhaps with the use of instruments specifically designed to assess pain related to Fabry's disease and quality-of-life issues.

In general, the infusions were well tolerated, and all 58 patients completed the double-blind trial and entered the open-label study. The possibility of infusion-related reactions was anticipated, since patients with classic Fabry's disease have no detectable α -galactosidase A activity, protein, or both.¹ Therefore, we purposely kept the infusion rates slow to maintain blinding, and we administered prophylactic medications to all patients to minimize any infusion-related reactions. During the open-label study, we increased the infusion rates, and in the case of many patients, the infusion lasted two hours. In 88 percent of patients, IgG antibodies against recombinant α -galactosidase A developed; however, seroconversion did not affect primary or secondary efficacy results, nor did the antibodies have a neutralizing effect, as occurs in patients with hemophilia A in whom inhibitors develop.^{18,19} After approximately one year of treatment with recombinant α -galactosidase A, IgG titers had decreased in 58 percent of patients with seroconversion and became undetectable in one patient. On the basis of previous experience with long-term enzyme-replacement therapy,^{10,20} such findings suggest that immunologic tolerance may develop in these patients.

In conclusion, we found that a dose of 1 mg of recombinant α -galactosidase A per kilogram every other week for about six months to one year safely and effectively reversed the accumulation of microvascular endothelial deposits of globotriaosylceramide in the kidneys, heart, and skin. Continued treatment may be required to reduce the deposition of glycosphingolipids in other types of cells, to which less enzyme is delivered,¹⁷ particularly renal tubular epithelial cells, podocytes, and cardiomyocytes. Further experience will determine effective regimens for initial reversal and subsequent control of the accumulated glycosphingolipids in the capillary endothelium and other types of cells.

Supported in part by a Merit Award from the National Institutes of Health (5 R37 DK34045), by grants from the National Institutes of Health (5 M01 RR00071 and 5 M01 RR00425, to the General Clinical Research Centers

at the Mount Sinai School of Medicine and Cedars-Sinai Medical Center, and 5 P30 HD28822, to the Mount Sinai Child Health Research Center), and by a grant from Genzyme Corporation.

Dr. Desnick has received grant support from and serves as a consultant to Genzyme.

We are indebted to the patients who participated in the study and to the outstanding nursing staffs of the General Clinical Research Centers at all the investigational sites.

APPENDIX

In addition to the authors, the following members of the International Collaborative Fabry Disease Study Group participated in the study: **Investigators** — M. Banikazemi, J. Ibrahim, and A.P. Cheng (New York); L.J. Raffle (Los Angeles); P. Cochat (Lyons, France); M. Azizi and X. Jeune-maitre (Paris); A. Vellodi (London); J.E. Wraith (Manchester, United Kingdom); C.J. Chaves, K.B. Kanis, I. Linfante, and R. Llinas (Boston); D.K. Bosman, H.S.A. Heymans, C.E.M. Hollak, and F.A. Wijburg (Amsterdam); **Expert pathologists** — *Kidney*: R.B. Colvin (Boston); S. Dikman (New York), and H. Rennke (Boston); *Heart*: H.T. Aretz (Boston), J. Fallon (New York), and R. Mitchell (Boston); *Skin*: H.R. Beyers and S. Granler (Boston) and R. Phelps (New York); and *General Pathology*: R.E. Gordon (New York); **Specialty consultants** — S. Brodie, S.A. Gass, M. Goldman, D. Mehta, and J. Winston (New York); R. Bouvier, B.P. Denis, L. Dubourg, A. Fouilhoux, A. Hadj-Aissa, M. Laville, I. Maire, B. Ranchin, and M.T. Vanier (Lyons, France); A. Hickey, J. Jordan, S. Jordan, S.S. Khan, and E. Maguen (Los Angeles); C. Amrein, B. Diebold, J.N. Fiessinger, M. Froissart, J.P. Grunfeld, J. Julien, L.H. Noel, C. Orssaud, and L. Poenaru (Paris); M.H. Griffiths, D. Holdright, N. Phelps-Brown, S. Sporton, R. Woolfson, V.C. Worthington, and E.P. Young (London); M. Bhushan, A. Cooper, E. O'Riordan, R. Radford, S.G. Ray, and R.S. Reeve (Manchester, United Kingdom); F.G. Berson, M.S. Kruskal, and W.J. Manning (Boston); W.J.W. Bos, D.K. Bosman, F.J.W. ten Kate, R.T. Krediet, K.I. Lie, J.J. Piek, L.J.J.M. Prick, and J.H.S. Smitt (Amsterdam); **Study coordinators and nurses** — M. Nunn, A. Nieto, R.A. Denchy, and A. Kowalski (New York); J. Exantus, M.T. Dupret, S. Garnier, and S. Walblich (Lyons, France); A.G. Verne and B. Williams (Los Angeles); M.C. Bernard and V. Remones (Paris); J. Morrison, D.G. Burke, L.G. Fulford, M. Jackson, R. Lobó, S. Sporton, and V.C. Worthington (London); B.M. Kenny (Manchester, United Kingdom); L. Baron (Boston); A. Vyth (Amsterdam); **Genzyme personnel** — R. Moscicki, T. Braakman, M. Goldberg, M. O'Callaghan, R. Cintron, S. Richards, P.K. Tandon, M.A. Fitzpatrick, M. Yelmene, and M. Nichols.

REFERENCES

- Desnick RJ, Ioannou YA, Eng CM. α -Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The metabolic and molecular bases of inherited disease*. 8th ed. Vol. 3. New York: McGraw-Hill, 2001:3733-74.
- Mapes CA, Anderson RL, Sweeley CC, Desnick RJ, Krivit W. Enzyme replacement in Fabry's disease, an inborn error of metabolism. *Science* 1970;169:987-9.
- Brady RO, Tallman JF, Johnson WG, et al. Replacement therapy for inherited enzyme deficiency: use of purified ceramidetrihexosidase in Fabry's disease. *N Engl J Med* 1973;289:9-14.
- Desnick RJ, Dean KJ, Grabowski G, Bishop DF, Sweeley CC. Enzyme therapy in Fabry disease: differential in vivo plasma clearance and metabolic effectiveness of plasma and splenic α -galactosidase A isozymes. *Proc Natl Acad Sci U S A* 1979;76:5326-30.
- Schiffmann R, Murray GJ, Treco D, et al. Infusion of alfa-galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. *Proc Natl Acad Sci U S A* 2000;97:365-70.
- Eng CM, Banikazemi M, Gordon RE, et al. A phase 1/2 clinical trial of enzyme replacement in Fabry disease: pharmacokinetic, substrate clearance, and safety studies. *Am J Hum Genet* 2001;68:711-22.
- Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W. Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes: α -galactosidase activities in plasma, serum, urine, and leukocytes. *J Lab Clin Med* 1973;81:157-71.
- Desnick RJ, Dawson G, Desnick SJ, Sweeley CC, Krivit W. Diagnosis of glycosphingolipidoses by urinary-sediment analysis. *N Engl J Med* 1971;284:739-44.
- Zeidner KM, Desnick RJ, Ioannou YA. Quantitative determination of globotriaosylceramide by immunodetection of glycolipid-bound recombinant verotoxin B subunit. *Anal Biochem* 1999;267:104-13.
- Richards SM, Olson TA, McPherson JM. Antibody response in pa-

tients with Gaucher disease after repeated infusion with macrophage-targeted glucocerebrosidase. *Blood* 1993;82:1402-9.

11. Melzack R. The short-form McGill Pain Questionnaire. *Pain* 1987;30:191-7.
12. Ware J, Snow K, Kosinski M, Gandek B. SF-36 Health Survey: manual and interpretation guide. 2nd ed. Boston: Health Institute, New England Medical Center, 1997.
13. Grabowski GA, Leslie N, Wenstrup R. Enzyme therapy for Gaucher disease: the first 5 years. *Blood Rev* 1998;12:115-33.
14. Mistry PK. Gaucher's disease: a model for modern management of a genetic disease. *J Hepatol* 1999;30:Suppl 1:1-5.
15. Elleder M, Bradova V, Smid F, et al. Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease: report on a case simulating hypertrophic non-obstructive cardiomyopathy. *Virchows Arch A Pathol Anat Histopathol* 1990;417:449-55.
16. von Scheidt W, Eng CM, Fitzmaurice TF, et al. An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med* 1991;324:395-9.
17. Ioannou YA, Zeidner KM, Gordon RE, Desnick RJ. Fabry disease: preclinical studies demonstrate the effectiveness of α -galactosidase A replacement in enzyme-deficient mice. *Am J Hum Genet* 2001;68:14-25.
18. Goodeve AC, Williams I, Bray GL, Peake IR. Relationship between factor VIII mutation type and inhibitor development in a cohort of previously untreated patients treated with recombinant factor VIII (Recombinate). *Thromb Haemost* 2000;83:844-8.
19. Lusher JM, Arkin S, Abildgaard CF, Schwartz RS, Kogenate Previously Untreated Patient Study Group. Recombinant factor VIII for the treatment of previously untreated patients with hemophilia A: safety, efficacy, and development of inhibitors. *N Engl J Med* 1993;328:453-9.
20. Rosenberg M, Kingma W, Fitzpatrick MA, Richards SM. Immunosurveillance of alglucerase enzyme therapy for Gaucher patients: induction of humoral tolerance in seroconverted patients after repeated administration. *Blood* 1999;93:2081-8.

Copyright © 2001 Massachusetts Medical Society.

Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy

BETH L. THURBERG, HELMUT RENNKE,¹ ROBERT B. COLVIN,¹ STEVEN DIKMAN,¹
RONALD E. GORDON, A. BERNARD COLLINS, ROBERT J. DESNICK, and MICHAEL O'CALLAGHAN

Departments of Pathology and Preclinical Biology, Genzyme Corporation, Cambridge, and Department of Pathology, Brigham and Women's Hospital, and Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts; Departments of Pathology and Human Genetics, Mount Sinai School of Medicine, New York, New York, USA

Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy.

Background. Fabry disease, a lysosomal storage disease caused by deficient lysosomal α -galactosidase A activity, is characterized by globotriaosylceramide (GL-3) accumulation in multiple cell types, particularly the vasculature, leading to end organ failure. Accumulation in the kidney is responsible for progressive decline in renal function in male patients with the classical phenotype, resulting in renal failure in their third to fifth decades of life. With the advent of recombinant protein synthesis technology, enzyme replacement therapy has become a viable alternative to dialysis or renal transplantation, previously the only available treatment options for end-stage renal disease.

Methods. The pre- and post-treatment renal biopsies were analyzed from fifty-eight Fabry patients enrolled in a Phase 3 double-blind, randomized, placebo-controlled trial followed by a six-month open label extension study of the recombinant human enzyme, α -galactosidase A (r-h α GalA), administered IV at 1 mg/kg biweekly. The purpose of this investigation was to detail the pathologic changes in glycosphingolipid distribution and the pattern of post-treatment clearance in the kidney.

Results. Baseline evaluations revealed GL-3 accumulations in nearly all renal cell types including vascular endothelial cells, vascular smooth muscle cells, mesangial cells and interstitial cells, with particularly dense accumulations in podocytes and distal tubular epithelial cells. After 11 months of r-h α GalA treatment there was complete clearance of glycolipid from the endothelium of all vasculature as well as from the mesangial cells of the glomerulus and interstitial cells of the cortex. Moderate clearance was noted from the smooth muscle cells of arterioles and small arteries. Podocytes and distal tubular epithelium also

demonstrated evidence for decreased GL-3, although this clearance was more limited than that observed in other cell types. No evidence of immune complex disease was found by immunofluorescence despite circulating anti-r-h α GalA IgG antibodies.

Conclusions. These findings indicate a striking reversal of renal glycosphingolipid accumulation in the vasculature and in other renal cell types, and suggest that long-term treatment with r-h α GalA may halt the progression of pathology and prevent renal failure in patients with Fabry disease.

Fabry disease is an X-linked recessive disorder in which affected males are deficient in the lysosomal enzyme α -galactosidase A. This deficiency leads to accumulation of neutral glycosphingolipids, mainly globotriaosylceramide (GL-3, also referred to as Gb3, ceramide trihexoside, CTH), in tissues throughout the body. Progressive glycosphingolipid accumulation results in clinical disease, primarily in affected males, but some female heterozygotes may be symptomatic depending on the pattern of Lyonization [1–3]. In affected males with the classical phenotype who have absent or very low levels of α -galactosidase A activity, angiokeratomas, hypohidrosis, acroparesthesias, transient ischemic attacks and stroke, congestive heart failure, cardiac conduction abnormalities, myocardial infarction, and progressive renal failure are the major clinical manifestations of the disease [4]. Atypical variants with residual α -galactosidase A have a milder phenotype with manifestations limited to the heart (that is, the cardiac variant [5, 6]). Of note, most cardiac variants do not have endothelial glycosphingolipid deposition, do not develop renal failure and live a normal lifespan, but usually die of the late cardiac manifestations of the disease. In contrast, progressive renal failure in affected males with the “classic” form of Fabry disease limits their average lifespan to the early forties, in the absence of dialysis or renal transplantation [7, 8]. Until the recent advent of recombinant enzyme

¹Dr. Rennke, Dr. Colvin, and Dr. Dikman contributed equally to this study.

Key words: renal disease, Fabry disease, X-linked recessive disorder, neutral glycosphingolipids, renoprotection, end-stage renal disease, Phase 3 trial.

Received for publication March 22, 2002
and in revised form June 24, 2002
Accepted for publication July 22, 2002

© 2002 by the International Society of Nephrology

replacement therapy [9], little could be done to combat the underlying pathology of Fabry disease, particularly the progressive renal disease and vascular pathology responsible for the early demise of most patients. Recently, a Phase 1/2 trial [9] and a Phase 3 trial with an open label extension [10] were conducted to evaluate the safety and efficacy of recombinant human α -galactosidase A (r-h α GalA) replacement therapy. These trials demonstrated that r-h α GalA replacement completely cleared GL-3 inclusions from the renal peritubular (interstitial) capillary endothelium (the primary endpoint of the Phase 3 trial) and effected equally remarkable clearance from the capillary endothelium of other key tissues (heart, skin and liver). Clearance of GL-3 from the plasma, urinary sediment and homogenized kidney tissue also was demonstrated. The identity of GL-3 and its clearance from these samples were demonstrated by light microscopic, immunohistochemical and biochemical analyses [9, 10]. The endothelial end points for these Fabry trials were chosen based on evidence implicating underlying vascular injury as the primary cause of the secondary kidney pathology such as global glomerulosclerosis and interstitial fibrosis [11, 12].

Vascular injury has been implicated in organ damage to the skin, heart, liver and CNS [4, 13]. The vascular endothelium plays an important normal physiologic role in glomerular perfusion, filtration, and maintenance of a continuous anti-coagulant vascular lining. Injury to endothelial cells in other disease settings leads to vasoconstriction within the glomerulus, and intravascular inflammation and thrombosis of vessels in the renal cortex and medulla, causing renal injury and failure [14–17] as well as complications such as accelerated atherosclerosis and thromboembolism [18]. More recently, investigators have surveyed Fabry patients and confirmed the elevation of markers of endothelial cell injury and activation [intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin], leukocyte activation (CD11b) and coagulation [tissue plasminogen activator (tPA), von Willebrand factor (vWF), plasminogen activator inhibitor (PAI)] [11, 13]. These abnormalities suggest that the vascular endothelial cells of Fabry patients are in a chronic pro-inflammatory and pro-thrombotic state [11, 13]. Loss of nephrons from vascular injury leads to increased pressures on remaining nephrons, leading to further accelerated nephron injury. Clearance of GL-3 from the endothelium by r-h α GalA, therefore, is expected to alleviate ongoing vascular damage and prevent progressive perfusion deficits that would otherwise contribute to renal failure in these patients.

The primary emphasis of the Phase 3 clinical trial was on establishing the significance of the vascular endothelial endpoints and monitoring numerous clinical and safety parameters [10]. A substantial effort also focused on characterizing the pathologic patterns of GL-3 accumulation

in the kidneys of these Fabry patients and documenting the response of the various cell and tissue types to r-h α GalA treatment. Although a number of case reports have described the main morphologic characteristics of renal pathology and glycolipid accumulation in renal tissue from Fabry patients [2, 19], little information is available on the patterns of pathology among a larger group of patients and the response of the cell types containing GL-3 to recombinant enzyme therapy. Here, based on a comprehensive analysis of the relevant renal cell types, we report on the clearance characteristics of each cell type in response to r-h α GalA (1 mg/kg biweekly). The pattern of response in the different renal cell types provides insight into treatment strategies that are likely to succeed in improving the overall health of the kidney. The findings are encouraging for the long-term benefits of r-h α GalA replacement therapy in patients with Fabry disease.

METHODS

Patients and study design

The study population comprised 58 Fabry patients (56 males, 2 females) enrolled in a five-month Phase 3, double-blind, randomized, placebo-controlled trial (29 patients in the treatment and placebo arms, mean age \pm SD, 28.4 ± 11.4 and 32.0 ± 9.4 years, respectively). This was followed by an additional six-month open label extension study, in which all 58 patients received enzyme replacement therapy. Entry criteria required that patients be 16 years of age or greater, with native plasma α -galactosidase A levels less than 1.5 ng/mL, and baseline serum creatinine levels less than or equal to 2.2 mg/dL. Recombinant α -galactosidase A (r-h α GalA; agalsidase beta; Fabrazyme; Genzyme, Cambridge, MA, USA) at a dose of 1 mg per kilogram of body weight or placebo (phosphate-buffered mannitol) was administered intravenously at a rate of 0.25 mg per minute, every two weeks. Complete sets of three diagnostic quality renal biopsies taken per protocol at baseline, five months and 11 months, were available for 48 of the 58 patients. The remaining ten patient sample sets were available but were limited to one or two of the three time points.

Light microscopy

Preliminary studies revealed that the lipid was best demonstrated for light microscopy in one-micron, epon embedded sections. Therefore, renal biopsies were fixed in 3% glutaraldehyde in 0.2 mol/L sodium cacodylate buffer, pH 7.3, followed by post-fixation in 1% osmium tetroxide in 0.2 mol/L sodium cacodylate (Electron Microscopy Sciences, Fort Washington, PA, USA). The tissue was infiltrated overnight, and then embedded in a 1:1 mixture of Epon 811 A and B (Electron Microscopy Sciences), plus DMP-30/Propylene Oxide (Electron Mi-

croscopy Sciences). One micron sections were stained with a 1:1 mixture of Methylene Blue in 1% sodium borate and 1% Azure II (Fisher Scientific, Fairlawn, NJ, USA).

Scoring of light microscopy

Each renal biopsy was reviewed under light microscopy by three independent renal pathologists who were blinded to treatment status of the patient at the time of biopsy. All pathologists participated in preliminary sessions to establish the criteria for the grading scale used to evaluate baseline GL-3 content and subsequent clearance from affected tissues. Cell types evaluated included: peritubular (interstitial) capillary endothelial cells (the primary endpoint of the Phase 3 trial), glomerular endothelial cells, mesangial cells, arterial/arteriolar endothelial cells, vascular smooth muscle cells, interstitial cells (a mixed cell population of fibroblasts and phagocytic cells), podocytes, and the epithelium of distal convoluted tubules and collecting ducts. The mesangial cell matrix of individual glomeruli was also evaluated for pathologic change. Scoring of these elements was conducted according to the design shown in Table 1. For each cell type listed, a majority score (the score common to at least 2 of the 3 evaluating pathologists) was derived from the individual scores of the three pathologists. When there was no common score for the primary endpoint (peritubular endothelial cells), the pathologists were reconvened for an adjudication. They reviewed the slide together at a multi-headed scope to reach a consensus score. For other cell types, the median score was selected. The distribution of these scores is summarized in Figure 1 and Table 2. Figure 1 shows the distribution of the means of all scores available at each time point. Table 2 shows the distribution of the score shifts from baseline. The differences in the "N" values between these two images are due to different presentation of the data. In some instances, certain cell types were absent from individual biopsy samples, and could not be evaluated at all time points. To allow for the variation occurring among glomeruli, the mesangial cell matrix for each individual glomerulus in the biopsy samples (to a maximum of 8) received a score ranging from 0 to 2. Individual glomerular scores were then averaged for each pathologist. The mean for the three pathologists' scores was then calculated.

Electron microscopy

Thin sections were cut from the tissue blocks prepared above and stained with 5% uranyl acetate (in a 1:1 mixture, methanol:water) and a modified Reynold's Lead Citrate. Photographs were taken at $\times 3300$ and $\times 10,000$ magnification using a JEM 100CX electron microscope (JEOL, Ltd, Tokyo, Japan).

Table 1. Scoring system for GL-3 accumulation/clearance in different renal cell types

Cell/tissue	Score
Glomerular endothelial cells	0 = None or trace accumulation
Peritubular capillary endothelial cells	1 = Mild accumulation 2 = Moderate accumulation
Arterial/arteriolar endothelial cells	3 = Severe accumulation
Vascular smooth muscle cells	
Podocytes ^a	
DCT/collecting ducts ^a	
Mesangial cells: GL-3 accumulation	0 = No lipid granules 1 = Minimal lipid granules
Interstitial cells	2 = Numerous lipid granules
Mesangial matrix	0 = Normal mesangium 1 = Mild expansion 2 = Moderate expansion

^aFor the podocytes and distal convoluted tubules (DCT)/collecting ducts, an increase (+), a decrease (-) or no change (0) response was collected for all post-baseline scores by comparing the baseline biopsy to a 5 or 11 month biopsy. Therefore, there are no absolute baseline values for these cell types, only relative values at 5 months and 11 months that reflect a comparison to the baseline.

Immunofluorescence

Immunofluorescence studies also were performed to determine whether IgG antibodies against r-h α GalA, which developed in most patients receiving the enzyme, had resulted in immune complex deposition in the kidney. Frozen tissue samples from five r-h α GalA-treated patients who developed IgG antibodies by the five month biopsy (IgG+ Group), three r-h α GalA-treated patients who did not develop IgG antibodies at five months (IgG- Group), and five untreated placebo patients (Control Group) were analyzed. Pre-treatment, baseline samples were available from two of these patients (one IgG+ and one IgG-). Four-micron sections from fresh frozen blocks of renal biopsies were stained with IgG fractions of fluorescein-conjugated monospecific antisera to human IgG, IgA, IgM, C3, C1q, fibrin/fibrinogen, and albumin (Dako Corp., Carpinteria, CA, USA) by standard clinical techniques [20]. Frozen sections were cut in a cryostat at -20°C and placed on Fisher Superfrost Plus slides (Fisher Scientific) and then air-dried. Using a Tissue-Tek II slide staining unit, slides were placed on a clinical rotator and pre-washed for five minutes in 0.01 mol/L sodium-phosphate-buffered saline (PBS), 0.15 mol/L NaCl, pH 7.3. One drop (50 to 100 μL) of diluted fluorescein conjugate was applied to the tissue section. The optimal concentration for each conjugate was determined by titrating on known positive and negative control tissues. Slides were then incubated in a moist, level, covered chamber for 30 minutes at room temperature. After incubation, the slides were washed in PBS for five minutes $\times 4$. The excess PBS was blotted from around the specimen, and the moistened tissue was coverslipped with Aquamount

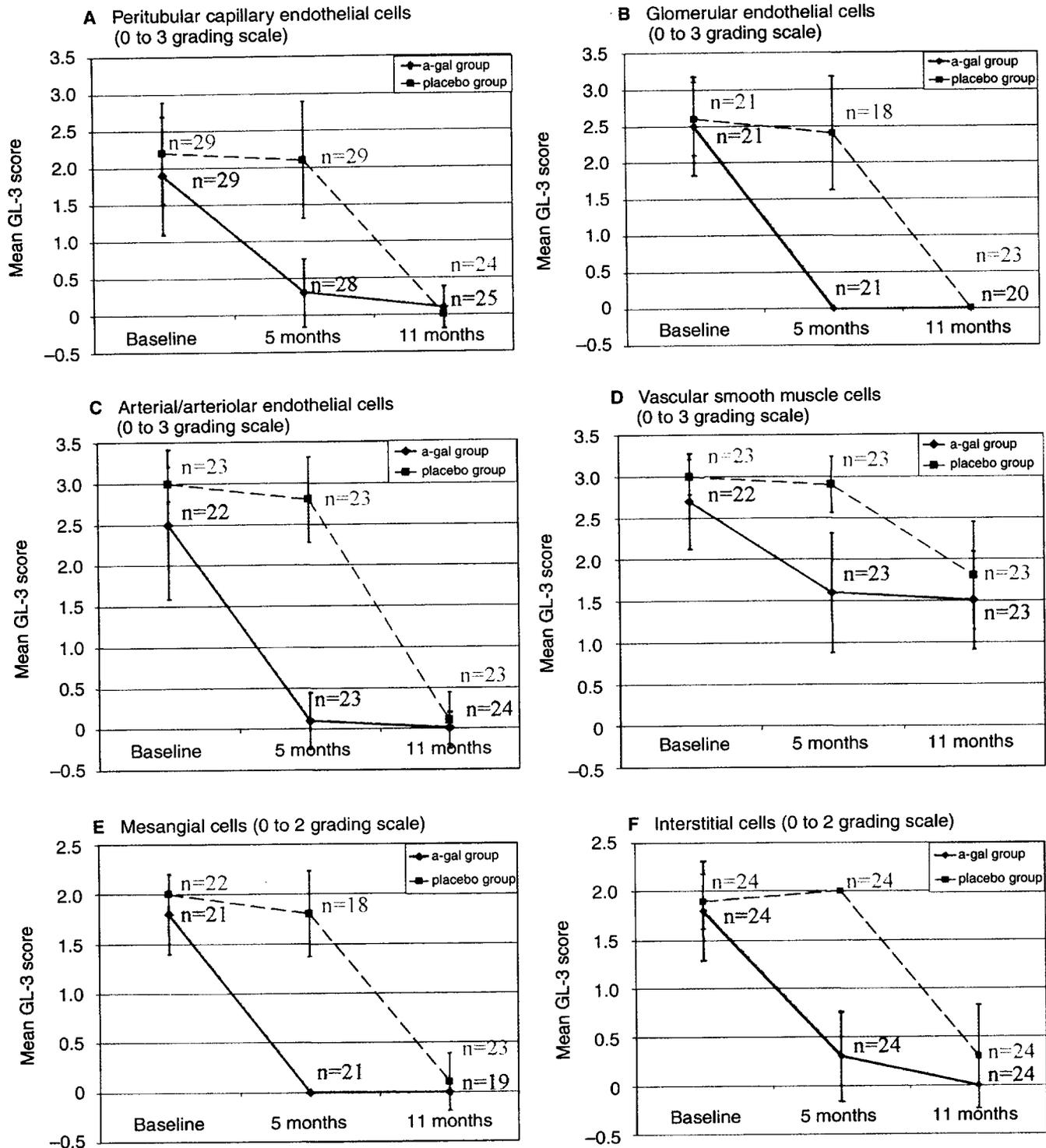


Fig. 1. Mean globotriaosylceramide (GL-3) scores with standard deviations were calculated for each cell type above in both the treatment groups at baseline, 5 months and 11 months. Note the rapid decline in mean GL-3 scores for the placebo group once enzyme replacement therapy was initiated after the 5-month time point. Treatment groups maintained their low GL-3 scores from the pivotal study (baseline to 5 months) into the extension segment of the study (evaluated at 11 months). (A) Peritubular capillary endothelial cells; (B) glomerular capillary endothelial cells; (C) arterial/arteriolar endothelial cells; (D) vascular smooth muscle cells; (E) mesangial cells; (F) interstitial cells. (Note: The differences in the "N" values between Figure 1 and Table 2 are due to different presentation of the data. In some instances, certain cell types were absent from individual biopsy samples, and could not be evaluated at all time points. Hence, the shift data presented in Table 2 have lower "N" values in some cases.)

Table 2A. Percentage of patients achieving zero scores* (percentages of patients with a 1–3 point score reduction, including all zero scores, are shown in parentheses)

Renal cells evaluated	TX group	Score shift from baseline to 5 months			Score shift from baseline to 11 months		
		Baseline	5 months	N	Baseline	11 months ^a	N
Peritubular capillary endothelial cells	Placebo	0%	0% (38%)	29	0%	100% (100%)	24
	A-gal	3%	69% (86%)	29	0%	92% (96%)	25
Glomerular capillary endothelial cells	Placebo	0%	0% (31%)	16	0%	100% (100%)	21
	A-gal	0%	100% (100%)	19	0%	100% (100%)	17
Mesangial cells	Placebo	0%	0% (19%)	16	0%	90% (100%)	21
	A-gal	0%	100% (100%)	19	0%	100% (100%)	17
Interstitial cells	Placebo	0%	0% (0%)	24	0%	78% (91%)	24
	A-gal	4%	71% (100%)	24	4%	100% (100%)	24
Arterial/arteriolar endothelial cells	Placebo	0%	0% (19%)	22	0%	87% (100%)	22
	A-gal	10%	86% (100%)	21	9%	96% (100%)	22
Vascular smooth muscle cells	Placebo	0%	0% (10%)	22	0%	0% (86%)	22
	A-gal	0%	10% (86%)	21	0%	0% (81%)	21

* The clearance of GL-3 from multiple renal cell types was determined before and after enzyme replacement therapy. There were two groups of patients: (1) the placebo group received placebo for the first 5 months and then α -galactosidase A for the remaining 6 months and (2) the A-gal group which received α -galactosidase A for the entire 11 months. The percentage of patients who attained a zero score at each biopsy time point is displayed and the percentage of all patients who demonstrated a reduction in score, including all zeros, is in parentheses. Values are presented for both the placebo and treatment groups. For statistical purposes, it was necessary for each patient to have both a baseline score and a shift score (5 months or 11 months) for each cell type, in order to be included in this data set. Two baseline columns are presented; the "N" designates the number of paired scores available for each cell type (paired scores meaning baseline and 5 months, or baseline and 11 months). This permitted calculation of the statistical significance of the change in score from either baseline to 5 months, or from baseline to 11 months, when comparing the placebo and treated groups. This distinction was necessary because (1) some patients had a poorly preserved or missed biopsy at one of the time points, and (2) some biopsy specimens did not contain glomeruli and therefore those cells could not be evaluated at all time points.

Table 2B. Percentage of patients achieving a reduction in GL-3 relative to baseline*

Renal cells evaluated	TX group	5 months		11 months ^a	
		%	N	%	N
Tubular epithelium	Placebo	4%	24	78%	24
	A-gal	25%	24	50%	24
Podocytes	Placebo	0%	16	23%	22
	A-gal	5%	19	18%	17

* Tubular epithelium and podocyte clearance were expressed as the percentage of patients attaining a relative reduction in the 5 month and 11 month biopsy. An increase (+), a decrease (-) or no change (0) response was collected for all post baseline scores by comparing the baseline biopsy to a 5 or 11 month biopsy. Therefore, there are no absolute baseline values for these cell types, only relative values at 5 months and 11 months that reflect a comparison to the baseline. A-gal represents patients treated with the recombinant human enzyme, α -galactosidase A.

^a Placebo group has crossed over to treatment at this time point

and glycerol (Lerner Labs, Pittsburgh, PA, USA). Coded slides were scored blinded, without knowledge of treatment groups or antibody status, using an epi-illumination fluorescence microscope. The intensity and distribution of glomerular staining was scored from 0 to 4 for each of the antibodies.

RESULTS

Baseline biopsies

At baseline, GL-3 accumulation in the kidney tissues was extensive, but varied considerably in quantity and morphology among the different cell types (Fig. 2). Podocytes and distal tubular epithelial cells contained the highest concentrations of GL-3 inclusions, whereas proximal tubular epithelial cells were relatively unaffected. The appearance of the lipid inclusions also varied, appearing in some cell types as small, dark, dense beaded granules, and in others as larger, complex, laminated bodies (myelin figures).

Globotriaosylceramide accumulation in vascular endothelial cells appeared as small, dense, beaded, peri-

nuclear cytoplasmic inclusions. GL-3 also accumulated in severely affected cells as larger grouped inclusions in the peripheral cytoplasm. The larger cytoplasmic inclusions often clustered and protruded into the lumens of small capillaries (Figs. 3A and 4A). On electron microscopy many of these granules were dense and amorphous (Fig. 3C). Vascular smooth muscle cells also accumulated moderate amounts of granular, cytoplasmic GL-3 (Fig. 5A). GL-3 in mesangial cells appeared as dense granules clustered around the nucleus in the cytoplasm (Fig. 6A). Interstitial fibroblasts and phagocytic cells ("interstitial cells") of the renal cortex also shared a similar appearance (Fig. 7A).

Lipid accumulation in podocytes and the epithelial cells of distal convoluted tubules and collecting ducts was much more concentrated and extensive than the accumulation present in endothelial cells, smooth muscle cells, mesangial cells or interstitial cells. Podocyte nuclei were often eccentrically positioned, pushed aside by the mass of accumulated GL-3 inclusions in the form of multiple scroll-like myelin figures filling the entire cytoplasm (Fig. 8A). The laminated appearance was particularly promi-

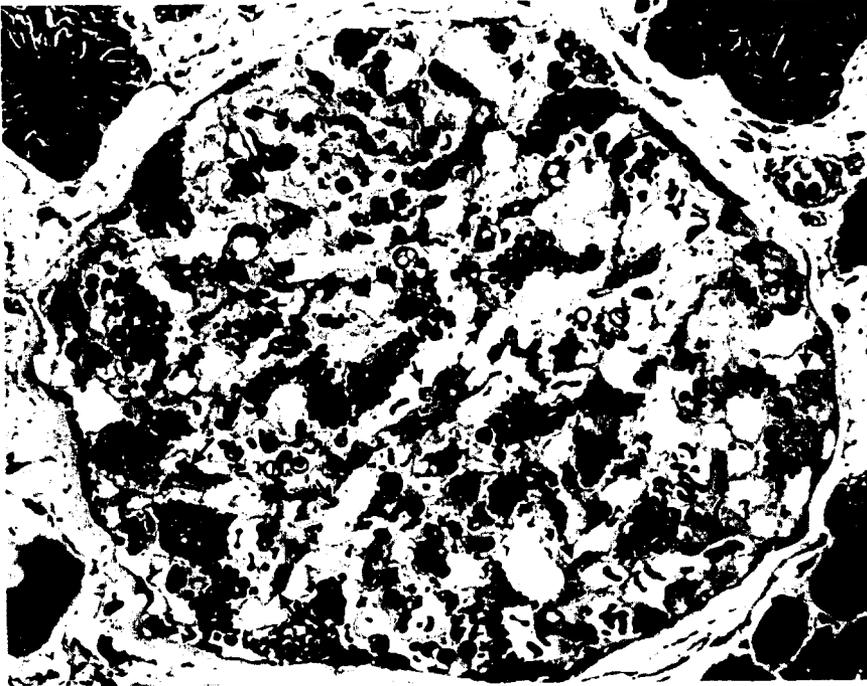


Fig. 2. GL-3 accumulates as dark blue granules and scroll-like whorls in multiple cell types of the renal glomerulus. Red arrows indicate endothelial accumulation; yellow arrows indicate mesangial cell accumulation; green "P" indicates podocyte accumulation (Magnification, $\times 40$ objective).

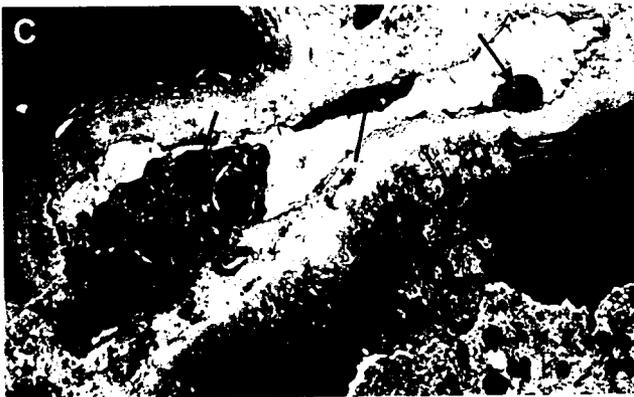
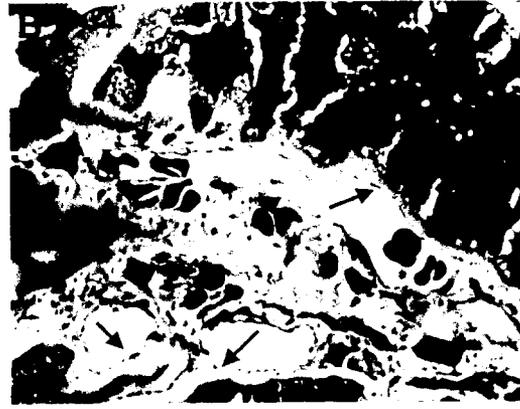
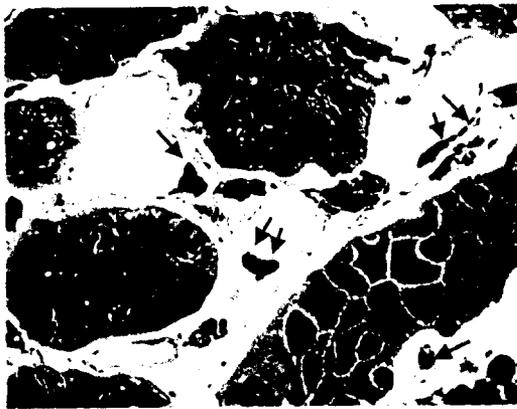


Fig. 3. GL-3 is cleared from endothelial cells of the peritubular (interstitial) capillaries. (A) Baseline biopsy, pre-treatment. GL-3 is present near endothelial nuclei and around the perimeter of the capillaries. (B) Post-treatment biopsy. Capillaries are clear of GL-3 (magnification of A and B, $\times 100$ objective). (C) Electron microscopic image of capillary endothelial cell GL-3 accumulations (arrows) protruding into the lumen, at baseline (Magnification, $\times 3300$).

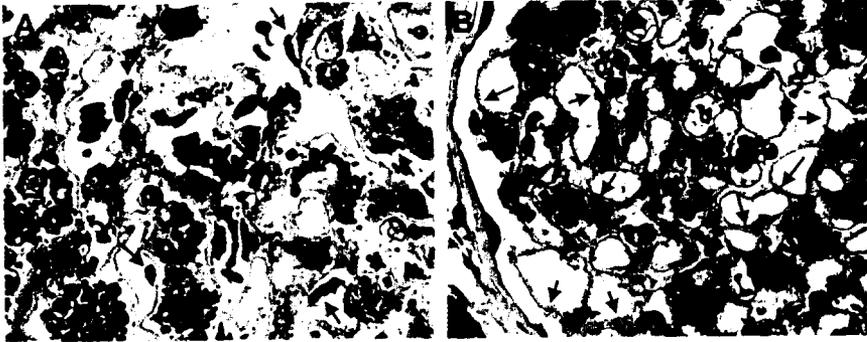


Fig. 4. GL-3 is cleared from endothelial cells of the glomerular capillaries. (A) Baseline biopsy, pre-treatment. Note how large clusters of GL-3 granules protrude into the vascular lumens of the glomerular capillaries. (B) Post-treatment biopsy. Protruding GL-3 clusters have been completely removed from the glomerular capillary lumens (magnification, $\times 100$ objective).

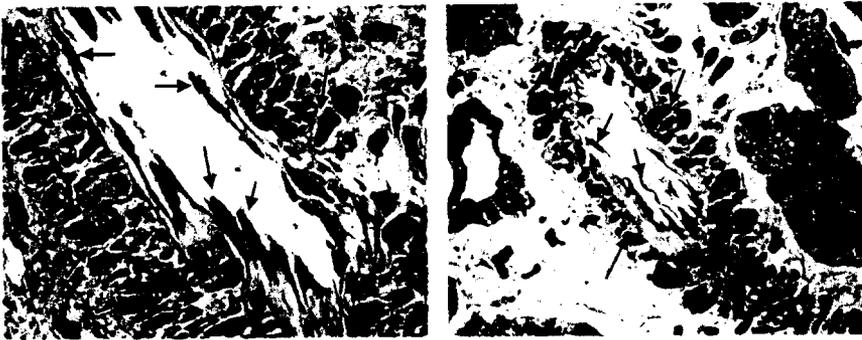


Fig. 5. GL-3 is cleared from endothelial cells (black arrows) and smooth muscle cells (red arrows) of renal arterioles. (A) Baseline biopsy, pre-treatment. Note how GL-3 accumulates in very large clusters in endothelial cells of these larger vessels. (B) Post-treatment biopsy. Both endothelial cells and smooth muscle cells of this vessel have achieved significant clearance of GL-3 (magnification, $\times 100$ objective).

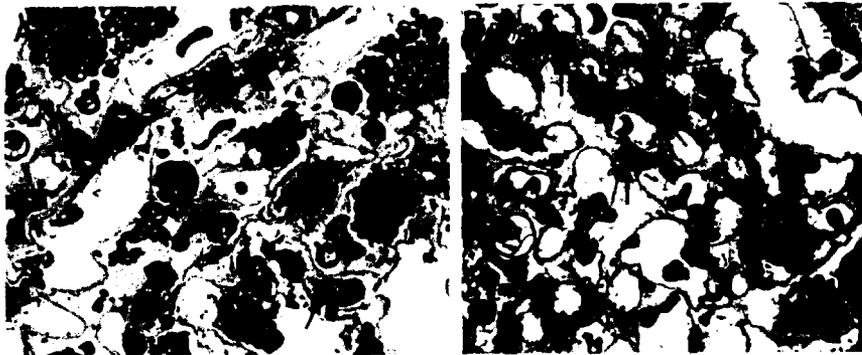


Fig. 6. GL-3 is cleared from mesangial cells. (A) Baseline biopsy, pre-treatment. Dense GL-3 granules accumulate around the nuclei of mesangial cells. (B) Post-treatment. Mesangial cells are completely cleared of GL-3 (magnification, $\times 100$ objective).

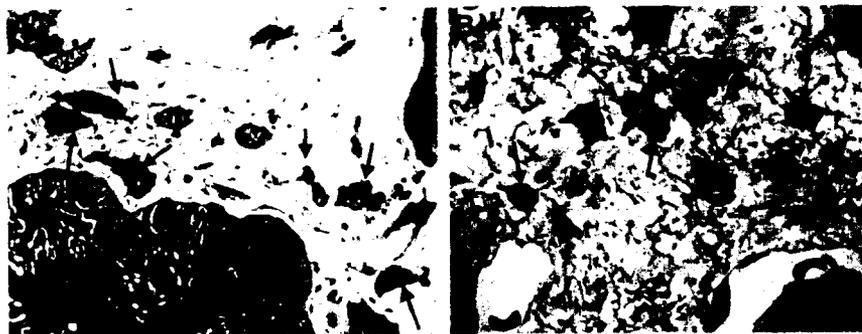


Fig. 7. GL-3 is cleared from interstitial cells. (A) Baseline biopsy, pre-treatment. GL-3 fills most of the cytoplasm of these interstitial cells. (B) Post-treatment. The interstitial cell cytoplasm has been cleared of GL-3 (magnification, $\times 100$ objective).

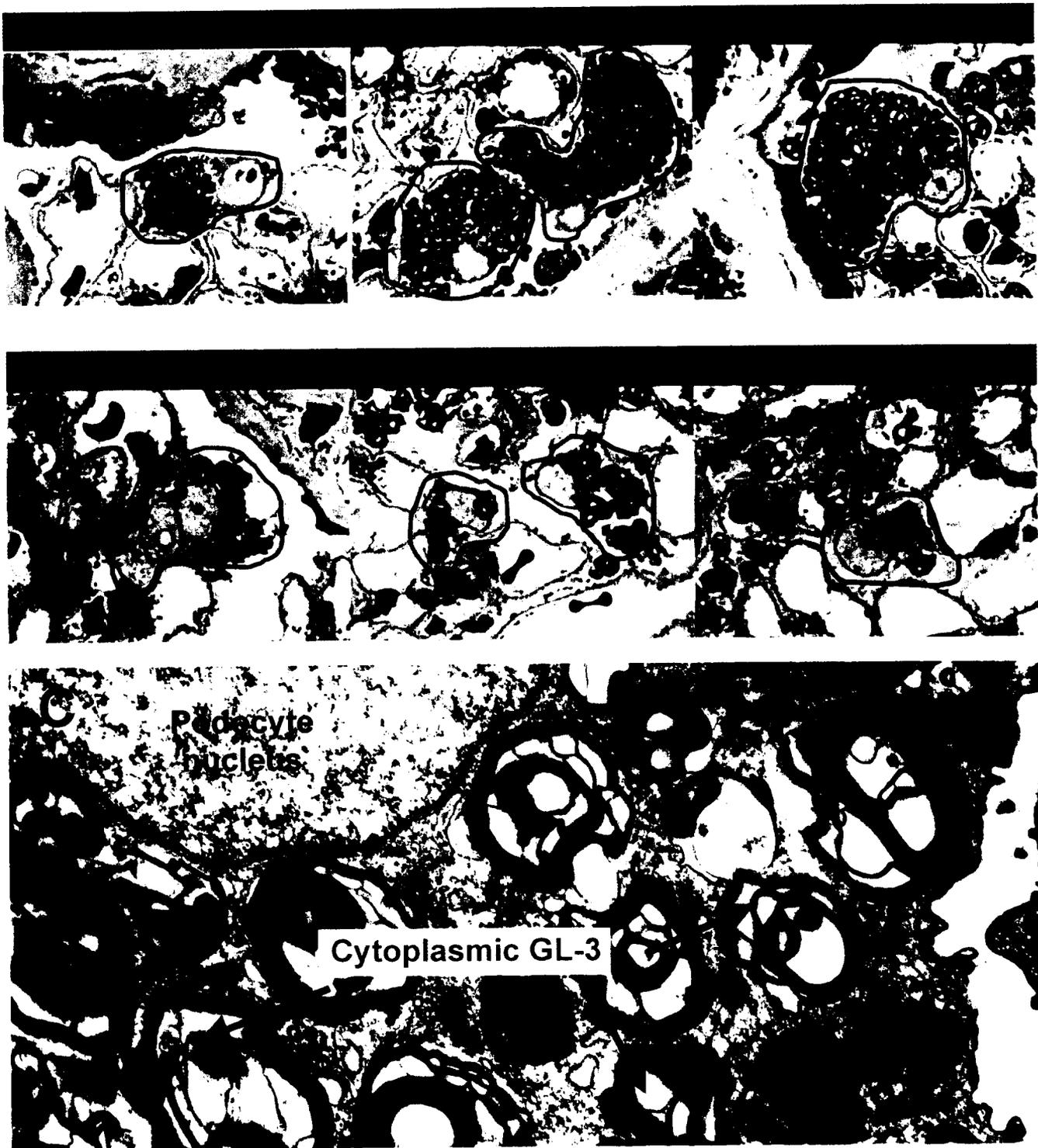


Fig. 8. GL-3 is cleared from podocytes. (A) Baseline biopsy, pre-treatment. GL-3 appears as multiple, tight scroll-like figures that fill the cytoplasm of each podocyte. (B) Post-treatment. The scroll-like GL-3 forms are looser and less numerous after treatment. Podocytes are outlined pre- and post-treatment for emphasis (magnification of A and B, $\times 100$ objective). (C) Electron microscopic image demonstrating the lamellar ultrastructure of the GL-3 accumulation in podocyte cytoplasm at baseline (magnification, $\times 10,000$).

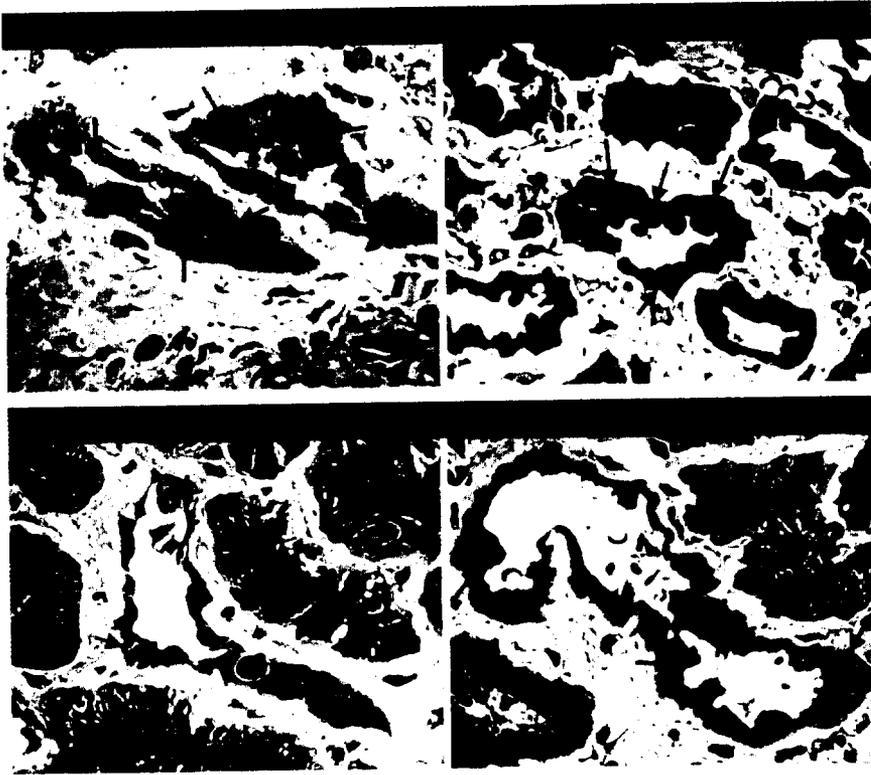


Fig. 9. GL-3 is cleared from tubular epithelium. (A) Baseline biopsy, pre-treatment. GL-3 appears as multiple small and large dense granules, some with a scroll-like architecture, within the epithelial cell cytoplasm of distal convoluted tubules and collecting ducts. (B) Post-treatment. The granules are fewer in number after treatment (magnification, $\times 100$ objective).

ment by electron microscopy (Fig. 8C). GL-3 accumulations in the epithelium of distal convoluted tubules and collecting ducts were similar in concentration and appearance to those in the podocytes (Fig. 9A).

Post-treatment biopsies

Vascular endothelium. Treatment with r-h α GalA resulted in statistically significant clearance of lipid from endothelial cells of all vessel types examined: peritubular capillaries, glomerular capillaries and arterial/arteriolar vessels.

In the patient group receiving r-h α GalA from baseline to five months, 86% demonstrated a reduction (or maintained a zero score) in GL-3 from peritubular capillary endothelial cells (Fig. 3B); 69% of patients achieved an endothelial score of zero (baseline mean = 1.9; five-month post-treatment mean = 0.3; Fig. 1A). After an additional six months of treatment, 96% of patients in this treatment group demonstrated a reduction in score (relative to baseline), with 92% achieving a score of zero (complete clearance), as demonstrated by biopsy at month 11. In the placebo group, 38% of patients demonstrated a reduction in GL-3 from baseline to five months; however, none (0%) achieved a score of zero (baseline mean = 2.2; five-month post-placebo mean = 2.1; Fig. 1A). There was a statistically significant difference between r-h α GalA and placebo-treated patients in the pla-

cebo-controlled (5 month) study ($P < 0.001$; based on a χ^2 test). After crossover to treatment for an additional six months, 100% (Table 2A) of the former placebo patients achieved an endothelial score of zero at month 11, demonstrating complete clearance ($P < 0.001$).

For glomerular capillary endothelial cells, patients receiving r-h α GalA from baseline to five months demonstrated 100% clearance of GL-3 (Fig. 4B), all attaining endothelial scores of zero (baseline mean = 2.5; five-month post-treatment mean = 0.0; Fig. 1B). All patients in this treatment group maintained a score of zero (complete clearance) after an additional six months of treatment. In the placebo group, 0% of patients attained zero endothelial scores from baseline to five months (baseline mean = 2.6; five-month post-placebo mean = 2.4; Fig. 1B). There was a statistically significant difference between the r-h α GalA and placebo treated patients in the placebo-controlled (5 month) study ($P < 0.001$). After crossover to treatment for an additional six months, however, 100% of the former placebo patients achieved endothelial scores of zero at month 11, demonstrating complete clearance ($P < 0.001$).

Examination of the arterial/arteriolar endothelial cells demonstrated that 100% of the r-h α GalA-treated patients had reduced GL-3 levels (Fig. 5B), with 86% achieving a score of zero from baseline to five months (baseline mean = 2.5; five-month post-treatment mean = 0.1; Fig.

1C). After an additional six months of treatment, these patients continued to improve, with 96% achieving a score of zero for complete clearance. In the placebo group, 19% of patients demonstrated a reduction in GL-3 score, however, none received a score of zero from baseline to five months (baseline mean = 3.0; five-month post-placebo mean = 2.8; Fig. 1C). There was a statistically significant difference in the percentage of zero scores between treated and placebo groups in the placebo-controlled study ($P < 0.001$). After the crossover to enzyme therapy for six months, 100% of the former placebo patients demonstrated a reduction in GL-3 score with 87% achieving zero scores ($P < 0.001$; Table 2A).

Vascular smooth muscle cells. Enzyme therapy removed GL-3 from vascular smooth muscle cells (Fig. 5B), although the clearance was less complete than that observed in other cell types. In the patient group treated with r-h α GalA from baseline to five months, 86% demonstrated a reduction in GL-3 score; however, only 10% received zero scores (baseline mean = 2.7; five-month post-treatment mean = 1.6; Fig. 1D). After an additional six months of treatment these values were relatively unchanged, as 81% demonstrated a reduction in GL-3 score and 0% had a score of zero. At this time point, reversal of zero scores occurred in two patients: one received a score of 1 and the other received a score of 2. In the placebo group, 10% of patients demonstrated a reduction in GL-3 score, however, none achieved a score of zero (baseline mean = 3.0; five-month post-placebo mean = 2.9; Fig. 1D). After crossover to treatment with r-h α GalA for an additional six months, 86% (Table 2A) demonstrated a reduction in GL-3 score and the mean score declined to 1.8 (Fig. 1D).

Mesangial cells. The lipid inclusions in mesangial cells of the renal glomeruli were cleared after five months of enzyme treatment (Fig. 6B). Scored on a scale from 0 to 2, the mesangial cells of all patients (100%) treated with r-h α GalA from baseline to five months demonstrated complete clearance (zero scores) of GL-3, and maintained their zero scores after an additional six months of r-h α GalA (baseline mean = 1.8; 5 and 11 month post-treatment means = 0.0; Fig. 1E). In the placebo group, 19% of patients showed a reduction in GL-3 scores, however, none achieved a score of zero from baseline to five months (baseline mean = 2.0; five month post-placebo mean = 1.8; Fig. 1E). There was a statistically significant difference in the percentage of zero scores between treated and placebo patients during the placebo-controlled study ($P < 0.001$). After crossover to treatment for an additional six months, 100% of the former placebo patients achieved a reduction in GL-3, with 90% (Table 2A) receiving a zero score indicating complete clearance of glycosphingolipid from the mesangial cells ($P < 0.001$).

Mesangial cell matrix. At baseline, both treatment groups

had moderate mesangial matrix widening (r-h α GalA-treated group, mean score = 0.8; placebo group, mean score = 0.9). There was no statistically significant difference between the mean scores from baseline to five months for the amount of matrix in either treatment group ($P = 0.284$). Change from baseline to 11 months showed a mean score decrease of only 0.1 for patients treated with the enzyme for the entire 11 months.

Interstitial cells. The lipid in interstitial cells was cleared after five months of treatment in the majority of patients (Fig. 7B). In the r-h α GalA cohort from baseline to five months, 100% demonstrated a reduction (or maintained zero scores) in GL-3 score, with 71% achieving complete clearance of interstitial cell GL-3 (baseline mean = 1.8; five-month post-treatment mean = 0.3; Fig. 1F). After an additional six months of treatment, 100% of the treatment group had achieved zero scores. In the placebo group, 0% achieved zero scores from baseline to five months (baseline mean = 1.9; five month post-placebo mean = 2.0; Fig. 1F). There was a statistically significant difference in the percentage of zero scores between treated and placebo patients in the placebo-controlled study ($P < 0.001$). After crossover to treatment for six months, 91% of the former placebo group demonstrated a reduction in GL-3 score, with 78% (Table 2A) achieving a score of zero ($P < 0.001$).

Epithelial cells: Podocytes and tubular epithelium. Podocytes responded to r-h α GalA enzyme therapy with a modest reduction in the numbers of lamellar GL-3 inclusions (Table 2B), and in many cases a more varied cytoplasmic GL-3 morphology, with some myelin figures being reduced to loose scroll-shaped bodies floating in the cell cytoplasm (Fig. 8B). In the r-h α GalA treated group, 5% showed a reduction in podocyte GL-3 after five months. This increased to 18% after an additional six months of treatment, demonstrating continued improvement of GL-3 clearance. In the placebo group, 0% of patients showed a reduction in GL-3 accumulation from baseline to five months. After crossover to treatment for an additional six months, 23% of patients began to show a relative reduction in podocyte GL-3 (Table 2B).

For the tubular epithelium, 25% of the samples in the r-h α GalA-treated group from baseline to five months demonstrated a relative reduction in GL-3 accumulation. After an additional six months of treatment in the extension study, 50% of these patients demonstrated a reduction in GL-3 (Fig. 9B). In the placebo group, only 4% demonstrated a reduction in GL-3 from baseline to five months; however, after crossover to r-h α GalA treatment for an additional six months, 78% of patients in this group demonstrated a relative reduction in tubular epithelium GL-3 (Table 2B).

Immunofluorescence for immune complex deposition. None of the patient samples tested (5 anti-r-h α GalA IgG positive, 3 anti-r-h α GalA IgG negative, 5 placebo, and

Table 3. Immunofluorescence result on glomerular deposits

Biopsy #	IgG anti-rhαGAL	IgG		IgA		IgM		C3		C1q		Fibrin/fibrogen	
		GBM	Mes	GBM	Mes								
Treated, with IgG titers	titer												
349	25600	0	0	0	0	0	0.5	0	0.5	0	0	0	0
359	25600	0.5	0	0.5	0.5	2	0.5	3	1	0	0.5	2	0
463	12800	0	0	0	0	0	0.5	0	0.5	0	0	0	0
73	12800	0	0	0	0	0	0.5	0	0.5	0	0	0	0
207	12800–25600	0	0	2	1	2	0.5	0	0.5	0	0	0	0.5
207 Pre	pre-treatment	0	0	0.5	1	2	1	0	0.5	0	0	0	0.5
Placebo with no IgG titers													
323		0	0	0.5	0.5	0	0.5	0	0.5	0	0	2	0.5
118		0	0	0	0	0	0.5	0	0.5	0	0	0.5	0.5
127		0	0	0.5	0.5	0	0.5	0	0.5	0	0	0.5	0.5
103		0	0	0	0	3	0.5	0	0.5	0	0	0.5	0.5
289		0	0	0	0	2	1	0	0.5	0	0	0	0.5
Treated, no IgG titers													
232		0	0	0	0	0.5	0.5	0.5	0.5	0	0	0	0
52		0	0	0	0	0.5	1	0	0.5	0	0	0.5	0.5
23		0	0	2	2	0.5	0	0.5	0.5	0	0	2	0.5
23 Pre	pre-treatment	0	0	1	2	2	1	0	0	0	0	0	0.5

Summary table of immunofluorescence results on immune complex deposition in the glomeruli of Fabry patients on Fabrazyme. Abbreviations are: GBM, glomerular basement membrane; Mes, mesangium. The intensity and distribution of glomerular staining were scored from 0 to 4 for each of the parameters listed. Note that IgG staining was negligible in all specimens examined for immune complex disease.

2 pre-treatment samples) had more than trace deposits of IgG in their glomeruli detected by immunofluorescence (Table 3). Two patients had IgA deposition, suggestive of IgA nephropathy (one in the IgG positive group and one in the IgG negative group). Pre-treatment samples were available for each of these patients and showed pre-existing IgA deposits. Three others had segmental IgM deposits typical of focal sclerosis (one IgG positive and two IgG negative patients). These findings indicate that despite five months of r-hαGalA treatment, patients with plasma IgG antibodies against the enzyme had no evidence of immune complex deposition in their kidneys.

DISCUSSION

The quantity of GL-3 that accumulates in different cell types in Fabry disease varies considerably. Some cells such as keratinocytes, proximal tubular epithelium and liver parenchyma are relatively devoid of lysosomal inclusions, whereas cardiomyocytes, podocytes and distal tubular epithelial cells are heavily laden. This cell-to-cell variation also is reflected in the total glycolipid content of different organs, in which certain cell types dominate. In the Phase I/II trial, mean whole tissue concentrations of GL-3 were 21,400 ng/mg in the heart, 5530 ng/mg in the kidney, 350 ng/mg in the skin and 1948 ng/mg in the liver [9]. The extremely high level in the heart reflects the predominance of the heavily laden cardiomyocytes in heart tissue, whereas the relatively low level in the liver reflects the dominance of parenchymal cells that were relatively free of GL-3. The kidney falls in the intermediate range since heavily laden cells such as podocytes and distal tubular epithelium are mixed with

cell types, such as endothelial cells, containing smaller glycolipid inclusions.

Variation in the amount of accumulated GL-3 among cells deficient in r-hαGalA is presumably determined by the amount of substrate generated by the cell type in question and the rate of cell turnover. The rate at which substrate is cleared will be determined by these same factors, plus the degree of exposure of the cell to the administered replacement enzyme. The ability of the enzyme to pass through various cellular and acellular barriers, such as the glomerular filtration apparatus, will determine its effectiveness at clearing substrate in different cell types. Fabrazyme (r-hαGalA) is a 110 kD dimeric glycoprotein containing mannose-6-phosphate terminated carbohydrates. It has been designed to target the cation-independent mannose-6-phosphate receptor (CIMPR), a receptor present on all normal mammalian cells. The CIMPR cycles continuously from the cell surface where it binds and endocytoses mannose-6-phosphate-containing ligands (such as newly synthesized lysosomal enzymes that have escaped sorting in the Golgi) to the lysosome, where these ligands are released [21, 22].

Of the renal cell types examined in this study, endothelial cells turn over the most frequently [23, 24] and, as demonstrated for the three vessel types examined (peritubular capillaries, glomerular capillaries, and arterioles and small arteries), were cleared of GL-3 within five months of initiating enzyme replacement treatment. Furthermore, as a consequence of intravenous delivery, the mannose-6-phosphate receptors on the surface of endothelial cells are likely to be exposed to the highest concentration of delivered enzyme. Thus, during the limited

50 to 100 day lifespan of endothelial cells [23], only a modest amount of GL-3 accumulates before the cell is replaced. The combination of rapid cell turnover and maximum exposure to r-h α GalA most likely explains the high degree of success in removing GL-3 from this key tissue within the first five months of treatment.

Mesangial cells also have a relatively rapid turnover rate [23] and demonstrated near complete GL-3 clearance within five months of initiating enzyme replacement therapy. The path of exposure of mesangial cells to r-h α GalA is unclear since the glomerular filtration barrier normally traps molecules greater than 69 kD. However, since the mesangial cells are in the immediate plasma filtration pathway of injured glomerular endothelium, which may allow passage of protein, mesangial cells are likely to have relatively high exposure to the enzyme. Immunohistochemical detection of the CIMPR on Fabry renal tissue has demonstrated that mesangial cells, as well as other affected renal cells, are rich in the receptor (Genzyme; unpublished observations). The interstitial cells accumulated small, dense GL-3 granules similar to those seen in endothelial and mesangial cells, and responded to enzyme replacement therapy to a similar degree. The interstitial cell population is comprised of fibroblast-like and phagocytic cells dispersed throughout the extra-glomerular interstitium. While there is little information on the turnover rate of this cell population, the rapid clearance of GL-3 observed suggests that regular cell turnover may play a role in the response of these cells.

The parietal epithelial cells (Bowman's capsule), followed by the visceral epithelial cells (podocytes) have the slowest turnover rate of the renal cell populations [23–25]. The tubular epithelium, however, is regularly shed in the urine and replaced, and has been shown to have a relatively high proliferation index [24]. This may account for the faster rate of GL-3 clearance following initiation of enzyme replacement therapy compared to that of podocytes. However, despite its high proliferation index, the tubular epithelium is more heavily laden with GL-3 than one might at first expect for a cell population that is regularly shed. Reports on the re-accumulation of GL-3 in normal kidneys transplanted into Fabry patients provide some insight into this apparent contradiction. One autopsy case reports the recurrence of Fabry disease in a transplanted kidney 11 years after transplant. Examination of the renal allograft revealed isolated accumulation of GL-3 in the endothelial cells of the capillaries [26]. It was suggested that high circulating levels of plasma GL-3 may overwhelm the enzymatic capacity of endothelial cells, which may accumulate GL-3 from the plasma by passive or endocytotic mechanisms. More importantly, a second autopsy case reported accumulation of GL-3 in both capillary endothelial cells and tubular epithelial cells in a renal allograft 14 years after transplant [27]. Urine is

concentrated along the distal convoluted tubules and collecting ducts. Therefore, it is probable that the epithelia of the distal convoluted tubules and collecting ducts are exposed to high concentrations of GL-3, leading to active or passive uptake. This may explain the marked accumulation of GL-3 observed in both this transplanted kidney and in the kidneys of all Fabry patients.

The similarities in the pattern and appearance of GL-3 accumulation in glomerular and tubular epithelia, despite their widely differing functions, are most likely a reflection of their common embryonic origin. The large quantity and organized ultrastructure of GL-3 in highly laminated myelin bodies in podocytes also is consistent with a lifetime of GL-3 accumulation due to untreated disease. Indeed, GL-3 accumulation has been observed in the renal epithelial cells of second trimester, hemizygous fetuses [28, 29], indicating early and active accumulation in these cells. Similar high concentrations of large, highly structured lysosomal inclusions are seen in cardiomyocytes, cells that are terminally differentiated with no regenerative capacity [30]. Given the large quantity of GL-3 in the renal epithelial cells, it is likely that implementation of enzyme replacement therapy over an extended period will be required to effect complete clearance. Since these cells are further down the filtration pathway they may suffer from less exposure to intravenously delivered enzyme. Nonetheless, there was good evidence from histologic examination of the podocytes and distal tubular epithelial cells that r-h α GalA had reduced the high baseline levels of GL-3 in these cells.

Numerous case reports suggest that while damage to podocytes, which play a role in the filtration of plasma proteins, may explain the presence of proteinuria in both hemizygotes and heterozygotes, this aspect alone cannot account for the precipitous renal failure in hemizygous male patients. Although the accumulation of GL-3 in podocytes of Fabry patients is dramatic, the renal pathology of some asymptomatic heterozygous females is often limited to significant accumulations of GL-3 in the podocytes with little alteration in renal function [1–3, 31]. One case report describes the inadvertent use of a heterozygous Fabry donor for transplantation to a genetically normal recipient. The graft was later found to contain numerous epithelial cell inclusions, but continued to function normally for 20 years [32, 33]. There is also a report of a 28-year-old male hemizygote with a subclinical variant of Fabry disease whose only renal pathology was podocyte accumulation of GL-3 [34]. This patient had a partial deficiency of α -galactosidase A. This finding suggests that while his enzyme level was not sufficient to prevent podocyte accumulation, it was enough to prevent clinical disease as seen in female heterozygotes. These cases and others [19, 35–39] suggest that residual enzyme activity, while perhaps not eliminating all signs of clinical or histologic disease, is still able to

significantly extend the patient's lifespan by obviating renal failure.

Most of the patients in the Genzyme-sponsored Phase III trial developed circulating IgG antibodies to r-h α GalA by the 3rd to 7th dose of enzyme [10]. However, the efficacy of r-h α GalA was not impaired by antibody development. This determination is based on the continued reduction in histologic scores for cellular clearance of GL-3 through the Extension study, even in patients who developed transiently higher titers [10]. As a safety measure, we also sought evidence of immune complex deposition in the kidney by immunofluorescence. Despite antibody titers of $\geq 12,800$ units in the five r-h α GalA-treated patients tested, no IgG deposits were found in their glomeruli. Segmental IgM was occasionally present, as expected in lesions with segmental scars, and was not correlated with anti-r-h α GalA antibodies. IgA deposition was detected in two treated patients (one with anti-r-h α GalA antibodies and one without). Similar IgA deposits were present in the pre-treatment biopsies of each patient, indicating that the process pre-dated r-h α GalA exposure.

Recent evidence that the endothelium of Fabry patients is in a pro-inflammatory and pro-thrombotic state [11, 13] supports the predominant role of vascular pathology in the pathogenesis of Fabry disease, including kidney failure [18]. Further evidence for the primary importance of the endothelial cells in the pathophysiology of Fabry disease comes from a comparison of the cardiac variant of Fabry disease with the classical form. Cardiac variant patients have marked GL-3 accumulation in cardiomyocytes, renal tubular epithelium and podocytes, as is seen also in classical Fabry patients, yet cardiac variants demonstrate little to no endothelial accumulation of GL-3. This difference suggests that the extended lifespan enjoyed by cardiac variant patients (relative to the shortened lifespan of classical Fabry patients) may be due to their lack of endothelial GL-3 accumulation. Male cardiac variants have residual enzyme activity, and this may be sufficient to keep endothelial cells clear of GL-3, thereby delaying the renal disease usually responsible for the death of the classical Fabry patient. The extended lifespan of the cardiac variant patient provides time for the GL-3 accumulation in the heart muscle to manifest itself clinically, as these patients often die later in life of cardiac disease [40]. Published case reports illustrate these points. The autopsy of a 63-year-old cardiac variant who died of conduction system abnormalities caused by glycosphingolipid accumulation, also was found to have GL-3 restricted to renal epithelial cells [41]. Clinically, the patient's renal decline was likely delayed by the residual enzyme activity. The absence of vascular endothelial cell involvement has been observed in other cardiac variants as well [42]. These cases suggest that it is not necessary to eliminate all signs of histologic disease to achieve significant clinical benefit by enzyme replacement ther-

apy. As mentioned earlier, complete clearance of a lifetime accumulation of GL-3 from some cell populations, such as cardiomyocytes and podocytes, is likely to require an extended treatment period. The most benefit would be afforded to those who begin treatment early in life, with the aim of arresting GL-3 cellular accumulation at sub-clinical levels. Residual enzyme activity in the cases described appears to maintain clearance of GL-3 from vascular endothelial cells and prevents the thrombosis-related injuries contributing to early morbidity and premature mortality in classical Fabry patients.

Clearance of GL-3 accumulation from cell types other than the vascular endothelium is likely to have considerable benefit for the overall health of the kidney by complementing the anti-inflammatory and anti-thrombotic benefits of vascular endothelial clearance. The mesangium, composed of cells and matrix, provides support to the glomerular capillary tuft. Mesangial cells have contractile properties that mediate filtration, and phagocytic properties that clean debris from the mesangium. Injury to mesangial cells primarily from intracellular GL-3 accumulation, and secondarily from capillary tuft vascular impairment, leads to the proliferation of cells and matrix, which in turn contributes to glomerulosclerosis [11]. During this study, we observed complete removal of GL-3 from mesangial cells and no change in mesangial cell matrix deposition. This observation may indicate either that treated mesangial cells cease or stabilize in their secretion of matrix, or that the deposition of matrix in Fabry disease is too slow to observe appreciable changes during an 11-month period.

The long-term treatment goal for Fabry patients is ultimately to prevent significant kidney pathology from developing by providing enzyme replacement therapy early in life. For older patients, the goal is to halt the progression of existing kidney pathology before the threshold of renal reserve is reached and life-threatening renal failure ensues. Given the effectiveness of r-h α GalA in clearing GL-3 from all of the relevant cell types of the kidney, even if slowly in podocytes and distal tubular epithelial cells, slowing the progression of disease in patients with early signs of renal failure also may be realistic. This pathology study has demonstrated that enzyme therapy with r-h α GalA resulted in significant clearance of the accumulated substrate from not only the critical endothelial compartment, but from all of the cell types involved in the renal pathology of Fabry disease, thereby providing a realistic means of stabilizing the disease and avoiding progression to clinical failure.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health including a research grant (R29 DK 34045 Merit Award), a grant (5 MO1 RR00071) for the Mount Sinai General Clinical Research Center Program from the National Center of Research

Resources, a grant (5 P30 HD28822) for the Mount Sinai Child Health Research Center, and a research grant from the Genzyme Corporation. Many thanks to the Genzyme Pathology Department staff for their help in developing a histologic grading system, to the Medical Information Department for their help with extensive literature searches on Fabry Disease, and to Biometrics for statistical data analysis. We also thank the clinical investigators and their patients at the multiple sites who participated in this trial: C.M. Eng, M. Banikazemi, J. Ibrahim, and A.P. Cheng (New York, NY, USA); W.R. Wilcox and L.J. Raffel (Los Angeles, CA, USA); N. Guffon and P. Cochat (Lyon, France); D.P. Germain, M. Azizi, and X. Jeunemaitre (Paris, France); P. Lee and A. Vellodi (London, UK); S. Waldek and J.E. Wraith (Manchester, UK); L. Caplan, C.J. Chaves, K.B. Kanis, I. Linfante, and R. Llinas (Boston, MA, USA); and C.E.M. Hollak, G.E. Linthorst, D.K. Bosman, H.S.A. Heymans, and F.A. Wijburg (Amsterdam, The Netherlands).

Reprint requests to Dr. Michael O'Callaghan, Department of Preclinical Biology, Genzyme Corporation, P.O. Box 9322, One Mountain Road, Framingham, Massachusetts 01701-9322, USA.
E-mail: mike.ocallaghan@genzyme.com

REFERENCES

- GUBLER MC, LENOIR G, GRUNFELD JP, et al: Early renal changes in hemizygous and heterozygous patients with Fabry's disease. *Kidney Int* 13:223-235, 1978
- FARGE D, NADLER S, WOLFE LS, et al: Diagnostic value of kidney biopsy in heterozygous Fabry's disease. *Arch Pathol Lab Med* 109: 85-88, 1985
- MARGUERY MC, GIORDANO F, PARANT M, et al: Fabry's disease: Heterozygous form of different expression in two monozygous twin sisters. *Dermatology* 187:9-15, 1993
- DESNIK RJ, IONNOU YA, ENG CM: α -Galactosidase A deficiency: Fabry disease, in *The Metabolic and Molecular Bases of Inherited Disease* (8th ed, vol 3), edited by SCRIVER CR, BEAUDET AL, SLY WS, VALLE D, New York, McGraw-Hill, pp 3733-3774, 2001
- VON SCHEIDT W, ENG CM, FITZMAURICE TF, et al: An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med* 324:395-399, 1991
- NAKAO S, TAKENAKA T, MAEDA M, et al: An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med* 333:288-293, 1995
- COLOMBI A, KOSTYAL A, BRACHER R, et al: Angiokeratoma corporis diffusum-Fabry's disease. *Helv Med Acta* 34:67-83, 1967
- WALLACE HJ: Anderson-Fabry disease. *Br J Dermatol* 88:1-23, 1973
- ENG CM, BANIKAZEMI M, GORDON RE, et al: A Phase 1/2 Clinical Trial of enzyme replacement in Fabry disease: Pharmacokinetic, substrate clearance, and safety studies. *Am J Hum Genet* 68:711-722, 2001
- ENG CM, GUFFON N, WILCOX WR, et al: Safety and efficacy of recombinant human α -galactosidase: A replacement therapy in Fabry's disease. *N Engl J Med* 345:9-16, 2001
- SAKURABA H, IGARASHI T, SHIBATA T, SUZUKI Y: Effect of vitamin E and ticlopidine on platelet aggregation in Fabry's disease. *Clin Genet* 31:349-354, 1987
- STERZEL RB, LOVETT DH: Interactions of inflammatory and glomerular cells in the response to glomerular injury, in *Immunopathology of Renal Disease. Contemporary Issues in Nephrology* (vol 18), edited by WILSON CB, BRENNER BM, STEIN JH, New York, Churchill Livingstone, 1988, pp 137-173
- DEGRABA T, AZHAR S, DIGNAT-GEORGE F, et al: Profile of endothelial and leukocyte activation in Fabry patients. *Ann Neurol* 47:229-233, 2000
- SAVAGE CO: The biology of the glomerulus: Endothelial cells. *Kidney Int* 45:314-319, 1994
- STEWART RJ, MARSDEN PA: Vascular endothelial cell activation in models of vascular and glomerular injury. *Kidney Int* 45(Suppl 45): S37-S44, 1994
- TAKANO T, BRADY HR: The endothelium in glomerular inflammation. *Curr Opin Nephrol Hypertens* 4:277-286, 1995
- NANGAKU M, SHANKLAND SJ, COUSER WG, JOHNSON RJ: A new model of renal microvascular injury. *Curr Opin Nephrol Hypertens* 7:457-462, 1998
- UTSUMI K, YAMAMOTO N, KASE R, et al: High incidence of thrombosis in Fabry's disease. *Intern Med* 36:327-329, 1997
- HULKOVA H, LEDVINOVA J, POUPETOVA H, et al: Postmortem diagnosis of Fabry disease in a female heterozygote leading to the detection of undiagnosed manifest disease in the family. *Cas Lek Cesk* 138:660-664, 1999
- COLLINS AB: Immunofluorescence, in *Diagnostic Immunopathology*, edited by COLVIN RB, BHAN AK, McCLUSKEY RT, New York, Raven Press, 1995, pp 699-710
- HILLE-REHFELD A: Mannose-6-phosphate receptors in sorting and transport of lysosomal enzymes. *Biochim Biophys Acta* 1141:177-194, 1995
- BRAULKE T: Type-2 IGF receptor: A multi-ligand binding protein. *Horm Metab Res* 31:242-246, 1999
- PABST R, STERZEL RB: Cell renewal of glomerular cell types in normal rat. An autoradiographic analysis. *Kidney Int* 24:626-631, 1983
- NADASYD T, LASZIK Z, BLICK KE, et al: Proliferative activity of intrinsic cell populations in the normal human kidney. *J Am Soc Nephrol* 4:2032-2039, 1994
- NAGATA M, NAKAYAMA K, TERADA Y, et al: Cell cycle regulation and differentiation in the human podocyte lineage. *Am J Pathol* 153:1511-1520, 1998
- MOSNIER JF, DEGOTT C, BEDROSSIAN J, et al: Recurrence of Fabry's disease in a renal allograft eleven year after successful renal transplantation. *Transplantation* 51:759-762, 1991
- GANTENBAIN H, BRUDER E, BURGER HR, et al: Recurrence of Fabry's disease in a renal allograft 14 years after transplantation. *Nephrol Dial Transplant* 10:287-289, 1995
- MALOUF M, KIRKMAN H, BUCHANAN P: Ultrastructure changes in antenatal Fabry's disease. *Am J Pathol* 82:13a, 1976
- TSUTSUMI A, SATO M, SATA K, et al: Early prenatal diagnosis of inborn error of metabolism: A case report of a fetus affected with Fabry's disease. *Asia Oceania J Obst Gynaecol* 11:39-45, 1985
- SOONPAA MH, DAUD AI, KOH GY, et al: Potential approaches for myocardial regeneration. *Ann N Y Acad Sci* 752:446-454, 1995
- RODRIGUEZ FH JR, HOFFMANN EO, ORDINARIO AT JR, BALIGA M: Fabry's disease in a heterozygous woman. *Arch Pathol Lab Med* 109:89-91, 1985
- GRUNFELD JP, LE PORRIER M, DROZ D, et al: Renal transplantation in patients suffering from Fabry's disease. Kidney transplantation from an heterozygote subject to a subject without Fabry's disease. *Nouv Presse Med* 4:2081-2085, 1975
- GRUNFELD JP, LIDOVE O, BARBEY F: Heterozygotes with Fabry's disease, in *Contributions in Nephrology* (vol 136), edited by SCHIEPPATI A, DAINA E, SESSA A, REMUZZI G, Basel, Karger, 2001, pp 208-210
- KAWAMURA O, SAKURABA H, ITOH K, et al: Subclinical Fabry's disease occurring in the context of IgA nephropathy. *Clin Nephrol* 47:71-75, 1997
- MIYASAKI K: Renal accumulation of glycosphingolipids. Report of a case and a review of literature. *Nephron* 14:456-465, 1975
- CHEN HC, TSAI JH, LAI YH, GUH JY: Renal changes in heterozygous Fabry's disease - A family study. *Am J Kidney Dis* 15:180-183, 1990
- SIVALOGANATHAN S: Fabry's disease - A rare cause of sudden death. *Med Sci Law* 32:263-266, 1992
- FUKUSHIMA M, TSUCHIYAMA Y, NAKATO T, et al: A female heterozygous patient with Fabry's disease with renal accumulation of trihexosylceramide detected with a monoclonal antibody. *Am J Kidney Dis* 26:952-955, 1995
- WUTHRICH RP, WEINREICH T, BINSWANGER U, et al: Should living related kidney transplantation be considered for patients with renal failure due to Fabry's disease? *Nephrol Dial Transplant* 13: 2934-2936, 1998
- YOSHITAMA T, NAKAO S, TAKENAKA T, et al: Molecular genetic, biochemical, and clinical studies in three families with cardiac Fabry's disease. *Am J Cardiol* 87:71-75, 2001
- IKARI Y, KUWAKO K, YAMAGUCHI T: Fabry's disease with complete atrioventricular block: Histological evidence of involvement of the conduction system. *Br Heart J* 68:323-325, 1992
- ELLEDER M, BRADOVA V, SMID F, et al: Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease. Report on a case simulating hypertrophic non-obstructive cardiomyopathy. *Virchows Arch A Pathol Anat* 417:449-455, 1990

STUDY REPORT

Epidemiologic Study of the Natural History of Fabry Disease

Study Number: AGAL-014-01

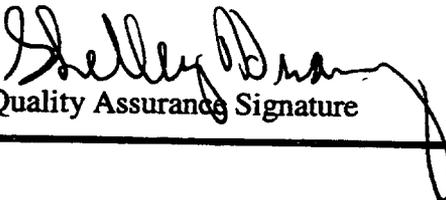
Final: 10 October 2002

Study Director: Donna Mackey, MS
Associate Director, Clinical Research
Genzyme Corporation
(617) 591-5859

Medical Monitor: Rekha Abichandani, MD
Associate Medical Director, Clinical Research
Genzyme Corporation
(617) 591-5741

Statistician: James MacDougall, PhD
Associate Director, Biomedical Operations
Genzyme Corporation
(617) 591-5995

This study was designed, conducted, recorded, and reported in compliance with the principles of Good Clinical Practice (GCP) guidelines. These guidelines are stated in U.S. federal regulations as well as "Guidance for Good Clinical Practice," International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.


Quality Assurance Signature

10/0ct/02
Date

2. SYNOPSIS

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Not Applicable NAME OF ACTIVE INGREDIENT Not Applicable	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
TITLE OF STUDY: Epidemiologic Study of the Natural History of Fabry Disease		
INVESTIGATORS AND STUDY CENTERS:		
Study Site #20 Maryam Banikanezi, M.D. Mount Sinai School Of Medicine New York, NY	Study Site #13 John Barranger, M.D. University of Pittsburgh Pittsburgh, PA	
Study Site #34 Daniel G. Bichet, MD Hopital du Sacre-Coeur de Montreal Montreal, Quebec, Canada	Study Site #51 Karl Brandspigel, M.D. 206 Hastings Lane Elizabeth City, NC	
Study Site #52 Daniel Brennan, M.D. 1 Barnes Hospital Plaza St. Louis, MO	Study Site #06 Jan Bultas, M.D., Ph.D. University Hospital Prague, Czech Republic	
Study Site #15 Louis Caplan, M.D. Beth Israel Deaconess Medical Ctr. Boston, Ma	Study Site #53 Alicia Chan, M.D. University of Alberta Hospital Edmonton, Alberta, Canada	
Study Site #16 Joel Charrow, MD Childrens Memorial Hospital Chicago, IL	Study Site #54 Joe Clarke, M.D., Ph.D., FRCP Hospital for Sick Children Toronto, Ontario, Canada	
Study Site #63 Sarah Dyack, MD IWK Health Care Halifax, Nova Scotia, Canada	Study Site #21 Louis Elsas, M.D Emory University Medical Genetics Atlanta, Ga	
Study Site #22 Christine Eng, MD Baylor College of Medicine Houston, TX	Study Site #41 Richard W. Erbe, M.D. UB School of Medicine and Biomedical Sciences Buffalo, NY	
Study Site #25 Richard Finkel, MD Children's Hospital of Philadelphia Philadelphia, PA	Study Site #24 Robert Hopkin, MD Childrens Hospital Medical Center Cincinnati, OH	

<p>NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139</p> <p>NAME OF FINISHED PRODUCT Not Applicable</p> <p>NAME OF ACTIVE INGREDIENT Not Applicable</p>	<p>SUMMARY TABLE Referring to Part of the Dossier:</p> <p>Volume:</p> <p>Page:</p> <p>Reference:</p>	<p>FOR NATIONAL AUTHORITY USE ONLY:</p>
<p>Study Site #9 Gabor Linthorst Academisch Medisch Centrum (AMC) Amsterdam, The Netherlands</p>	<p>Study Site #27 Seymour Packman, MD University of California San Francisco San Francisco, CA</p>	
<p>Study Site #57 Raphael Schiffmann, M.D. National Institute of Neurological Disorders and Stroke, NIH Bethesda, MD</p>	<p>Study Site #58 C. Schmitt, M.D. 3218 Nassau St. Everett, WA 98201-4139</p>	
<p>Study Site #30 Katherine B. Sims, M.D. Massachusetts General Hospital Boston, MA</p>	<p>Study Site #59 Dr. Sven Asger Sorensen Copenhagen, Denmark</p>	
<p>Study Site #48 Robert Steiner, M.D. Oregon Health Sciences University Portland, OR</p>	<p>Study Site #47 Janet Thomas, M.D. Children's Hospital Denver, CO</p>	
<p>Study Site #43 Neal Weinreb, M.D. Northwest Oncology/Hematology Assoc. 8170 Royal Palm Blvd. Coral Springs, FL</p>	<p>Study Site #32 William Wilcox, M.D., Ph.D. Cedars-Sinai Medical Center Los Angeles, CA</p>	
<p>Study Site #35 Philip Wyatt, M.D. North York General Hospital Toronto, Ontario, Canada</p>		
<p>PUBLICATION (REFERENCE): Not published</p>		
<p>STUDIED PERIOD: April 2001 – June 2002</p>		
<p>PHASE OF DEVELOPMENT: Not Applicable</p>		
<p>OBJECTIVES: The principal objectives of this study were:</p> <ul style="list-style-type: none"> • to characterize the natural history of Fabry disease • to estimate the rates of occurrence of renal disease, cardiac disease, cerebral vascular disease, 		

<p>NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139</p> <p>NAME OF FINISHED PRODUCT Not Applicable</p> <p>NAME OF ACTIVE INGREDIENT Not Applicable</p>	<p>SUMMARY TABLE Referring to Part of the Dossier:</p> <p>Volume:</p> <p>Page:</p> <p>Reference:</p>	<p>FOR NATIONAL AUTHORITY USE ONLY:</p>
<p>and/or death in a population of Fabry patients who have not received enzyme replacement therapy</p> <ul style="list-style-type: none"> • to provide a historical control for Genzyme r-hαGAL clinical trials • to characterize the hospitalization of patients with Fabry disease • to provide support for sample size calculations and conversion from placebo to historical control in Genzyme Study Number AGAL 008-00: Multi-center, Randomized, Double-Blind, Placebo-Controlled Study of the Safety and Efficacy of Recombinant Human α-Galactosidase A (r-hαGAL) on Progression of Renal Disease and Significant Clinical Events in Patients with Fabry Disease. 		
<p>METHODOLOGY:</p> <p>The basic study approach can be summarized as follows:</p> <ol style="list-style-type: none"> 1. Use of a historical cohort design that included 27 participating sites with patients who have a current diagnosis of Fabry disease or who had a diagnosis of Fabry disease at the time of death. 2. The sites obtained patients' informed consent and permission to release medical records. 3. Data were abstracted under the supervision of an independent contract research organization (Abt Associates Clinical Trials (AACT), 55 Wheeler Street, Cambridge, MA 02138) identified as an expert in methodologies of collecting epidemiologic and survey data. <p>All statistical analyses involving comparisons of the historical control study data to data from a prospective clinical trial will be specified in the study documentation of that prospective clinical trial.</p>		
<p>NUMBER OF PATIENTS (PLANNED AND ANALYZED):</p> <p>400 (planned); 447 analyzed (104 Qualified Patients – AGAL-008-00 inclusion/exclusion criteria)</p>		
<p>DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION/EXCLUSION:</p> <p>INCLUSION:</p> <p>Patients must meet each of the following criteria to be enrolled in this study:</p> <ol style="list-style-type: none"> 1. Current diagnosis of Fabry disease, or diagnosis of Fabry disease at time of death 2. Patient, guardian, or next of kin must consent to release medical records <p>EXCLUSION:</p> <p>Patients will be excluded from this study if they meet any of the following criteria:</p> <ol style="list-style-type: none"> 1. Patient had obvious and confounding renal disease (i.e., diabetic nephropathy, systemic lupus erythematosus, or other well-established disorder) at time of Fabry disease diagnosis 2. Patient had other major disease (i.e., cancer, HIV/AIDS) at time of Fabry disease diagnosis <p>THE AGAL-008-00 QUALIFIED POPULATION (N=104) WAS BASED ON THE INCLUSION/EXCLUSION CRITERIA OF THE AGAL-008-00 TRIAL (DESCRIBED BELOW):</p> <p>INCLUSION:</p> <p>Patients who met all of the following inclusion criteria were eligible for inclusion as AGAL-008-00 Qualified Patients:</p> <ol style="list-style-type: none"> 1. The patient must provide written informed consent prior to any study-related procedures being performed. 2. Patients must be ≥ 16 years old. 		

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Not Applicable NAME OF ACTIVE INGREDIENT Not Applicable	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
3. Patients must have a current diagnosis of Fabry disease. 4. Patients may not have received enzyme replacement therapy as treatment for Fabry disease. 5. Patients must have documented plasma αGAL activity of < 1.5 nmol/hr/mL or a documented leukocyte αGAL activity of < 4 nmol/hr/mg. 6. Patients must have one or more of the following: a. serum creatinine measurement ≥ 1.2 and < 3 mg/dL (106.1 and 265.2 μmol/L) b. estimated creatinine clearance < 80 mL/min (using the Cockcroft-Gault formula (Aronoff, 1995) if the patient's serum creatinine measurements < 1.2 mg/dL EXCLUSION: Patients who met any of the following exclusion criteria were not eligible for participation as AGAL-008-00 Qualified Patients: 1. Patient has undergone or is currently scheduled for kidney transplantation or is currently on dialysis. 2. Patient has acute renal failure. 3. Patient has unconfirmed Fabry disease. 4. Patient has normal αGAL activity. 5. Patient has the following: a. serum creatinine measurement of < 1.2 mg/dL (<106.1 μmol/L) (based on Screening period measurements) unless estimated serum creatinine clearance < 80 mL/min, OR b. history of transient ischemic attack (TIA) or ischemic stroke within 3 months of study entry. 6. Patient has received enzyme replacement therapy as treatment for Fabry disease. 7. Patient has diabetes mellitus or presence of confounding renal disease. 8. Patient has current critical coronary artery disease (as documented by a presently unstable angina and/or documented myocardial infarction within 3 months). 9. Patient has congestive heart failure (contributed by Fabry disease) as defined by Class III or Class IV cardiac status as evaluated under the New York Heart Association classification.		
TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION; BATCH NUMBER: Not Applicable		
DURATION OF TREATMENT: Not Applicable		
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION; BATCH NUMBER: Not Applicable		
CRITERIA FOR EVALUATION: SAFETY: Not Applicable EFFICACY: Primary study measurements were progression of renal disease. Progression of cardiac disease, cerebral vascular disease, and/or death was also evaluated. The evaluation for progression of renal disease was based upon those patients that met the		

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Not Applicable NAME OF ACTIVE INGREDIENT Not Applicable	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
<p>inclusion/exclusion criteria for the Phase 4 trial (AGAL-008-00). This population was referred to as the AGAL-008-00 Qualified patient population and comprised 104 patients. For other study measurements, the AGAL-008-00 Qualified patient population and overall population were evaluated.</p> <p>RENAL DISEASE</p> <p>Progression of chronic renal disease may be met by a 33% increase in serum creatinine levels from the date of the first available creatinine value greater than or equal to 1.2 mg/dL. In addition a 50% increase in serum creatinine was also evaluated.</p> <p>Progression to end stage renal disease as defined the need for chronic dialysis or transplantation.</p> <p>CARDIAC DISEASE</p> <p>Progression of cardiac disease may be met in one or more of the following ways:</p> <ul style="list-style-type: none"> • Myocardial infarction, as determined by the World Health Organization (WHO) standards specified in the Multinational Monitoring of Trends and Determinants in Cardio Vascular Disease (MONICA) manual • Significant change in cardiac status which requires new surgical/medical intervention • Arrhythmia requiring pacemaker, DC-cardioversion, implantation of a defibrillator, or drug intervention • Unstable angina • Worsening heart failure requiring hospitalization and intravenous medication to control symptoms <p>CEREBRAL VASCULAR DISEASE</p> <p>Progression of cerebral vascular disease may be met by direct observation of physical manifestations/clinical evidence of a new CVA or TIA.</p> <p>DEATH</p> <p>Death due to any cause</p>		
<p>STATISTICAL METHODS:</p> <p>SAFETY: Not Applicable</p> <p>EFFICACY:</p> <p>There are two components to the analyses: (1) analyses to provide a general characterization of the natural history of Fabry disease from disease onset or diagnosis until death and (2) analyses to provide a historical control for clinical trials of r-hαGAL.</p> <p>It should be noted that one of the key objectives for the analysis presented in this report is to serve as a historical control for the Phase 4 AGAL-008-00 study.</p> <p>Data were presented through data listings or summary tables. For tables, continuous outcomes were summarized with n's, means, standard deviations, medians, minimums, and maximums. Categorical variables were summarized with frequency counts and percentages. Graphical displays were presented as appropriate.</p>		

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Not Applicable NAME OF ACTIVE INGREDIENT Not Applicable	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:
---	---	--

For estimating the renal event rate the simplest method was employed, specifically empirical estimate based on tabulating the proportion of patients who were observed to have the event within a certain time period. The slope (rate of change) for serum creatinine will be estimated using regression methods.

For comparison to the AGAL-008-00 trial, more precise and robust estimates of the renal event incidence rate were obtained by modeling the underlying patient-specific trends in serum creatinine over the full duration of follow-up. The incidence rates, 80%, 84%, 90%, and 95% confidence intervals were computed for occurrence of renal disease using a linear random effects model based on serum creatinine values and estimated GFR over time. In addition, the results with covariates (age, gender, weight, blood type, and α-GAL levels) in the model were summarized.

Individual serum creatinine measurements were plotted over time along with the usage of ACE Inhibitors /ARBs-ACE Receptor Blockers and blood pressure measurements.

Time to occurrence was estimated for death using the Kaplan Meier methods. The estimated mortality rate was graphically displayed.

SUMMARY – CONCLUSIONS

SAFETY RESULTS:

Not Applicable

EFFICACY RESULTS:

RENAL

A total of 26/104 (25%) patients experienced renal events based on both the 33% and 50% increase in serum creatinine from Qualification Start Date and/or dialysis/transplant as defined in the study protocol.

Statistical modeling was performed to determine the event rates for the Qualified population based on AGAL-008-00 inclusion/exclusion criteria. These event rates would be used as a comparator to the predicted event rates from the patients enrolled in the AGAL-008-00 study to determine if treatment with r-hαGAL is effective in halting progression of renal disease in Fabry patients. For the Qualified Population (N=104), the estimated 2 and 3-year renal event rates based on a 33% and 50% increase, respectively, in serum creatinine values are presented in the Table 1.

Table 1

Serum Creatinine Increase	Estimated Event Rate
33% Increase Over 2 Years	30%
50% Increase Over 3 Years	32%
Reference: Table 14.8-3	

CARDIAC

A total of 49/104 (47%) patients in the AGAL-008-00 Qualified population experienced a cardiac event as defined in the study protocol.

NAME OF COMPANY Genzyme Corporation One Kendall Square Cambridge, MA 02139 NAME OF FINISHED PRODUCT Not Applicable NAME OF ACTIVE INGREDIENT Not Applicable	SUMMARY TABLE Referring to Part of the Dossier: Volume: Page: Reference:	FOR NATIONAL AUTHORITY USE ONLY:		
<p>CEREBROVASCULAR</p> <p>A total of 10/104 (10%) patients in the AGAL-008-00 Qualified population experienced a cerebrovascular event as defined in the study protocol.</p> <p>DEATH</p> <p>Seven of 104 (7%) patients meeting the AGAL-008-00 inclusion/exclusion criteria died during the time of their Qualification window. A total of 22/447 (5%) of patients in the overall study population died.</p> <p>CONCLUSION:</p> <p>It is proposed that the estimated 3-year renal event rate from the natural history study and corresponding confidence interval be used as a comparison to the Phase 4 study. The renal event rate of the single arm Phase 4 Fabrazyme® study will be determined to be different from the natural history study if their corresponding 84% confidence intervals are non-overlapping (Table 2).</p>				
<p>Table 2</p>				
<p>Estimated 3-yr Event Rate (50% increase in serum creatinine) in Historical Database AGAL-008-00 Qualifiers (N=104)</p>		<p>Projected 3-yr Event Rate (50% increase in serum creatinine) in AGAL-008-00 Patients (N=70; final projected enrollment)</p>		
<p>Event Rate</p>	<p>32%</p>	<p>10%</p>	<p>15%</p>	<p>20%</p>
<p>84% Confidence Interval</p>	<p>25, 36</p>	<p>5, 17</p>	<p>10, 23</p>	<p>13, 28</p>
<p>The confidence interval for the historical control event rate is based on the confidence interval for the slope parameter in the linear random effects model. The confidence interval for the projected event rate is based on a two-tailed Fisher-Exact test. Reference: Table 14.8-6</p>				

3. TABLE OF CONTENTS

1.	TITLE PAGE	1
2.	SYNOPSIS	2
3.	TABLE OF CONTENTS	9
4.	ABBREVIATIONS AND TERMS	17
5.	ETHICS	18
5.1	Independent Ethics Committee (IEC) or Institutional Review Board (IRB)	18
5.2	Ethical Conduct of the Study	18
5.3	Patient Information and Consent	18
6.	INVESTIGATORS AND STUDY ADMINISTRATION STRUCTURE	19
7.	INTRODUCTION	20
8.	STUDY OBJECTIVES	21
9.	INVESTIGATIONAL PLAN	22
9.1	Overall Study Design and Plan	22
9.2	Discussion of Study Design, Choice of Control Groups	22
9.3	Selection of Study Population	23
9.3.1	Inclusion Criteria: AGAL-014-01	23
9.3.2	Exclusion Criteria: AGAL-014-01	23
9.3.3	Inclusion Criteria: AGAL-008-00 Qualified Population	24
9.3.4	Exclusion Criteria: AGAL-008-00 Qualified Population	24
9.3.5	Removal of Patients from Therapy or Assessment	25
9.3.6	Patient Screening Log	25
9.4	Treatments	26
9.4.1	Treatments Administered	26
9.4.2	Identity of Investigational Product	26
9.4.3	Method of Assigning Patients to Treatment Groups	26
9.4.4	Selection of Doses in the Study	26
9.4.5	Selection and Timing of Dose for Each Patient	26
9.4.6	Blinding	27
9.4.7	Prior and Concomitant Therapy	27
9.4.8	Treatment Compliance	27
9.5	Efficacy and Safety Variables	27
9.5.1	Renal Disease	28
9.5.2	Cardiac Disease	28
9.5.3	Cerebrovascular Disease	29
9.5.4	Death	29
9.5.5	Efficacy and Safety Measurements Assessed and Flow Chart	29
9.5.6	Appropriateness of Measurements	29
9.5.7	Primary Efficacy Variable(s)	29
9.5.8	Drug Concentration Measurements	29
9.6	Data Quality Assurance	29
9.7	Statistical Methods Planned in the Protocol and Determination of Sample Size	29
9.7.1	Statistical and Analytical Plans	30
9.7.2	Efficacy Endpoint Data	30

9.7.2.1	Renal Endpoint Data.....	30
9.7.2.1.1	Progression of Renal Disease: Dialysis and Transplantation	30
9.7.2.1.2	Progression of Renal Disease for the AGAL-008-00 Qualified Population: Serum Creatinine.....	30
9.7.2.1.3	Estimated Glomerular Filtration Rate (GFR) using the MDRD Equation for the AGAL-008-00 Qualified Population	31
9.7.2.2	Cardiac Endpoint Data.....	32
9.7.2.3	Cerebrovascular Endpoint Data.....	32
9.7.2.4	Death Endpoint: Kaplan-Meier Estimate of Mortality Rate.....	32
9.7.2.5	Time to Events	33
9.7.3	Determination of Sample Size	33
9.7.4	Missing or Invalid Data	33
9.8	Changes in the Conduct of the Study or Planned Analyses.....	33
10.	STUDY PATIENTS.....	34
10.1	Disposition of Patients.....	34
10.1.1	Establishing the “Qualified” Population.....	34
10.1.2	Detailed Patient Disposition	35
10.2	Protocol Deviations.....	37
10.2.1	Deviations from Planned Analyses	37
11.	EFFICACY EVALUATION.....	38
11.1	Data Sets Analyzed.....	38
11.2	Demographic and Other Baseline Characteristics	38
11.3	Measurements of Treatment Compliance	43
11.4	Efficacy Results and Tabulations of Individual Patient Data	43
11.4.1	Summary of Events.....	43
11.4.2	Hospitalization of Patients in the Historical Database.....	44
11.4.3	Cardiac Events	45
11.4.4	Cerebrovascular Events	45
11.4.5	Death.....	46
11.4.6	Renal Events	47
11.5	Estimation of Renal Event Rates Based on Serum Creatinine and GFR Modeling in the AGAL-008-00 Qualified Population.....	48
11.5.1	Renal Event Modeling	48
11.5.2	Review of Renal Data	48
11.5.3	Historical Serum Creatinine Data	50
11.5.4	Renal Event Rate Modeling and Estimation.....	54
11.5.4.1	Event Rate Modeling and Estimation for the Qualified Population.....	54

11.5.4.2	Two- and Three-Year Event Rate Modeling and Estimation for the Qualified Population Stratified by Serum Creatinine Observations	55
11.5.4.3	Empirical Estimation of Renal Event Rates	58
11.5.4.4	Justification of the Logarithmic Transformation.....	60
11.5.4.5	Distribution of the Estimates Slopes and Intercepts.....	65
11.5.4.6	Adjustments for Covariates	67
11.5.4.7	By Subject Linear Regression	73
11.5.4.8	Likelihood Analysis.....	74
11.5.4.9	Subset Analyses	74
11.5.4.10	Analysis of 1 / Serum Creatinine.....	76
11.5.4.11	Historical Predicted Glomerular Filtration Rate.....	78
11.5.4.12	Handling of Dropouts or Missing Data	80
11.5.4.13	Interim Analyses and Data Monitoring	80
11.5.4.14	Multicenter Studies	80
11.5.4.15	Multiple Comparison/Multiplicity.....	80
11.5.4.16	Use of an “Efficacy Subset” of Patients	80
11.5.4.17	Active-Control Studies Intended to Show Equivalence.....	80
11.5.4.18	Examination of Subgroups	81
11.5.5	Tabulation of Individual Response Data	81
11.5.6	Drug Dose, Drug Concentration, and Relationships to Response	81
11.5.7	Drug-Drug and Drug-Disease Interactions	81
11.5.8	By-Patient Displays	81
11.5.9	Application of Natural History Data to Phase 4 Data and Justification of the Sample Size for the Phase 4 Study (AGAL-008-00).....	81
11.5.10	Efficacy Conclusions	82
12.	SAFETY EVALUATION.....	83
13.	DISCUSSION AND OVERALL CONCLUSIONS	84
14.	TABLES, FIGURES, AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT	85
14.1	Demographic Data	86
14.2	Efficacy Data	97
14.3	Safety Data.....	98
14.4	Summary of Events Data and Estimation of Event Rates	99
14.5	Summary of Tables from AGAL-008-00	202
15.	REFERENCE LIST.....	209
16.	APPENDICES.....	210
16.1	Study Information.....	211
16.1.1	Protocol and Protocol Amendments	212
16.1.2	Sample Case Report Form	328
16.1.3	Documentation of IRB and IEC Approval and Sample Patient Informed Consent Form.....	351

16.1.4	List and Description of Investigators and Other Important Study Directors	393
16.1.5	Signatures of Sponsor’s Responsible Medical Officers	782
16.1.6	Listing of Patients Receiving Test Drug from Specific Batches.....	783
16.1.7	Randomization Scheme and Codes.....	784
16.1.8	Audit Certificates.....	785
16.1.9	Documentation of Statistical Methods.....	791
16.1.9.1	Statistical Analysis Plan for Protocol Number: AGAL-014-01	792
16.1.9.2	Justification of 84% Confidence Interval	835
16.1.9.3	A Summary of the Statistical Methods and Planned Analyses for the Single Arm AGAL-008-00 Study as Compared to the AGAL-014-01 Historical Study	838
16.1.10	Documentation of Inter-Laboratory Standardization Methods and Quality Assurance Procedures	841
16.1.11	Publications Based on the Study.....	842
16.1.12	Important Publications Referenced in the Report.....	843
16.2	Patient Data Listings.....	844
16.2.1	Discontinued Patients	845
16.2.2	Protocol Deviations.....	846
16.2.3	Patients Excluded from the Efficacy Analysis	847
16.2.4	Demographic Data	848
16.2.5	Compliance and/or Drug Concentration Data	1575
16.2.6	Individual Efficacy Response Data.....	1576
16.2.7	Adverse Event Listings.....	1577
16.2.8	Listing of Individual Laboratory Measurements by Patient	1578
16.2.9	Listing of Individual Variables by Patient.....	2894
16.3	Case Report Forms.....	3804
16.4	Individual Patient Data Listings (US Archival Listings).....	3805

LIST OF IN-TEXT TABLES

Table 9-1:	Summary of Screening Logs.....	26
Table 10-1:	Summary of Inclusion/Exclusion of Patients.....	35
Table 10-2:	Summary of Patient Disposition Based on AGAL-008-00 Inclusion/Exclusion Criteria	36
Table 10-3:	Overall Study Duration and Study Duration by Time Groups for AGAL-008-00 Qualified Patients	37
Table 11-1:	Summary of Demographics of the Historical Patient Population at Fabry Disease Diagnosis	38
Table 11-2:	Summary of Demographics of the Qualified Historical Patient Population at Qualification Start Date Compared to the Phase 4 Study (AGAL-008-00) Population at Baseline.....	40

Table 11-3:	Interim Review: Summary of Fabry Disease History	41
Table 11-4:	Summary of Medical History	42
Table 11-5:	Summary of All Events	44
Table 11-6:	Mean Time (in Years) to Cardiac Event Occurrence in AGAL-008-00 Qualified Patients	45
Table 11-7:	Mean Time (in Years) to Cerebrovascular Event Occurrence in AGAL-008-00 Qualified Patients	46
Table 11-8:	Mean Time (in Years) To Death in AGAL-008-00 Qualified Patients	46
Table 11-9:	Mean Time (in Years) From Both Date of Birth and Qualification Start Date To Renal Event Occurrence in AGAL-008-00 Qualified Patients	48
Table 11-10:	Serum Creatinine Records.....	51
Table 11-11:	Populations Analyzed in Event Rate Estimation	51
Table 11-12:	Estimated Event Rate for AGAL-008-00 Qualified Population	56
Table 11-13:	Estimated Event Rate for Renal Events Based on AGAL-008-00 Qualified Population (n = 104) and the Random Effects Model	58
Table 11-14:	Estimates of Renal Event Rate Based on the Empirical Method	59
Table 11-15:	Estimates of Renal Event Rate Based on the Empirical Method (Centered Intervals).....	59
Table 11-16:	Linear, Quadratic, and Cubic Trend Random-Effects Model Fits.....	63

List of In-Text Tables, continued

Table 11-17:	Estimated Renal Event Rates Based on the Linear and Quadratic Trend Random-effects Models with Time-Subsets of Data.....	63
Table 11-18:	Parameter Estimates for Selected Covariates and Adjusted Renal Event Rate	68
Table 11-19:	Estimated Renal Events Rates Based on By-Subject Regressions.....	73
Table 11-20:	Estimated Renal Event Rate (50% Criteria) Based on the Linear Random Effects Model with Exclusion and Replacement Criteria	76
Table 11-21:	Estimated Renal Event Rate (33% Criteria) Based on the Linear Random Effects Model with Exclusion and Replacement Criteria	76
Table 11-22:	Estimated Renal Event Rates Using Logarithmic versus Reciprocal Transformations	78
Table 11-23:	Glomerular Filtration Rate (GFR) Records	79
Table 11-24:	Populations Analyzed in Event Rate Estimation	79
Table 11-25:	Estimated Event Rate for Qualified Population.....	79
Table 11-26:	Parameter Estimates for Selected Covariates and Adjusted Renal Event Rate	80
Table 11-27:	A Comparison of the Estimated Three-Year Event Rates from Historical Control Population versus the Projected Three-Year Event Rates from r-hαGAL (AGAL-008-00) Treated Patients	82

LIST OF IN-TEXT FIGURES

Figure 11-1: Kaplan-Meier Estimated Mortality Rate for Entire Study Population47

Figure 11-2: Reciprocal of Serum Creatinine Data (1/serum creatinine) From All AGAL-008-00 Qualified Patients52

Figure 11-3: Reciprocal of Serum Creatinine Data (1/serum creatinine) From All AGAL-008-00 Qualified Patients with Three or More Serum Creatinine Observations53

Figure 11-4: Longitudinal Data on Progression of Renal Insufficiency in Nine Patients with Fabry Disease (Branton, 2002, Medicine).....54

Figure 11-5: Distribution for the Slope of Log Creatinine for Qualified Patients (n = 104).....55

Figure 11-6: Distribution for the Slope of Log Creatinine for AGAL 008-00 Qualified Patients with Two or More Serum Creatinine Observations (n = 85).....56

Figure 11-7: Distribution for the Slope of Log Creatinine for AGAL-008-00 Qualified Patients with Three or More Serum Creatinine Observations (n = 64).....57

Figure 11-8: Distribution for the Slope of Log Creatinine for Patients with At Least One Serum Creatinine Measurement Between 1.2 – 3.0 mg/dL (n = 129)57

Figure 11-9: Distribution of Serum Creatinine for Qualified Patients over Time60

Figure 11-10: Distribution of the Log Serum Creatinine for Qualified Patients over Time61

Figure 11-11: Distribution of the Log of Serum Creatinine for Qualified Patients over First 5 Years of Observation Time62

Figure 11-12: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients66

Figure 11-13: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients with Two or More Serum Creatinine Records66

Figure 11-14: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients with Three or More Serum Creatinine Records67

Figure 11-15: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Plasma α-GAL.....69

List of In-Text Figures, continued

Figure 11-16: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Age at Qualification Quartiles69

Figure 11-17: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Serum Creatinine at Qualification Quartiles70

Figure 11-18: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Weight at Qualification Quartiles70

Figure 11-19: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Blood Type.....71

Figure 11-20: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Gender71

Figure 11-21: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by ACE Inhibitor Use.....72

Figure 11-22: Plot of Standardized Residuals for the 1/Creatinine Model versus Quantiles of Log Transformation and Reciprocal Transformation.....77

4. ABBREVIATIONS AND TERMS

αGAL	α-galactosidase A
r-hαGAL	recombinant human α-galactosidase A
AACT	Abt Associates Clinical Trials
ACE Inhibitors	Angiotensin Converting Enzyme Inhibitors
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ARBs	Angiotensin Receptor Blockers
CABG	Coronary Artery Bypass Graft
CPK	Creatine Phosphokinase
CPK-MB	Creatine Phosphokinase-Myocardial Muscle Band
CRA	Clinical Research Associate
CRF	Case Report Form
CVD	Cerebrovascular Disease
DCF	Data Clarification/Correction Form
ECG	Electrocardiogram
ERT	Enzyme Replacement Therapy
FDA	Food and Drug Administration (USA)
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GL-3	Globotriaosylceramide
HIV	Human Immunodeficiency Virus
IABP	Intra-Aortic Balloon Pump
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MDRD	Modification of Diet in Renal Disease Study Group
MI	Myocardial Infarction
NYHA	New York Heart Association
PTCA	Percutaneous Transluminal Coronary Angioplasty
QA	Quality Assurance
SAE	Serious Adverse Event
SIU	International Recommended System Units
TIA	Transient Ischemic Attack

5. ETHICS

5.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

This protocol, and the patient informed consent form were reviewed and approved by an Institutional Review Board (IRB) or Independent Ethics Committee (IEC) complying with the requirements of 21 CFR 50 and 56 and the International Conference on Harmonisation before enrollment of patients. The IEC at two international sites waived the need for review and approval because this was an epidemiological study. Genzyme Corporation received a copy of the letter of approval or waiver from the IRB or IEC and the approved consent form.

5.2 Ethical Conduct of the Study

This protocol was designed and conducted, recorded, and is reported in compliance with the principles of Good Clinical Practice (GCP) regulations established by the basic principles defined in the U.S. 21 CFR Part 312. These requirements are stated in “Guidance for Good Clinical Practice,” International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use.

5.3 Patient Information and Consent

Unless waived by the IEC, patients were required to read and sign an informed consent form summarizing the purpose of the study, the procedures to be carried out, and the potential hazards in non-technical terms. Patients were free to withdraw at any time for any reason or could have been withdrawn, if necessary, to protect their health or the integrity of the study. For deceased patients their next of kin were required to read and sign an informed consent form unless this was waived by the local IRB. A sample consent form can be found in Appendix 16.1.3.

6. INVESTIGATORS AND STUDY ADMINISTRATION STRUCTURE

Curriculum vitae for study personnel are included in Appendix 16.1.4.

7. INTRODUCTION

Fabry disease is an X-linked recessive inborn error of metabolism characterized by a deficiency in the lysosomal hydrolase, α -galactosidase A and occurs predominantly in males (*Desnick, 2001, McGraw-Hill*). The global incidence in males is approximately 1:40,000. However, heterozygote females may also develop pathology and symptoms due to unbalanced expression secondary to the random inactivation of the normal α -galactosidase gene (lyonization) (*Lyon, 1961, Nature*). In classically affected individuals, the phenotypic expression of Fabry disease is manifested as subnormal or absent activity of α -galactosidase A, resulting in the pathological accumulation of α -galactyl-terminated neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3) in cellular lysosomes. Accumulation occurs in virtually all tissues of the body, but particularly in the endothelial, perithelial, and smooth-muscle cells of blood vessels, the ganglion cells of the autonomic nervous system, the glomeruli and tubules of the kidney, and the cardiomyocytes of the heart. Although patients may not exhibit symptoms that would indicate involvement of these organ systems early in life, it has been established that the progressive accumulation of these substrates leads to dysfunction in these organ systems over time.

The natural history of the progression of renal function of Fabry patients has not been well-characterized. In addition, other relevant variables such as deterioration of cardiac function over time, occurrence of cerebrovascular events, and death rate due to Fabry disease progression has not been well characterized.

In April 2001, Genzyme reported on an historical database that permitted an estimate of the clinical progression of renal insufficiency in patients with Fabry disease. The limited data available in that historical database and the limited analyses and conclusions that could be drawn from the data highlighted the need for a rigorously compiled, broader historical database that could be used to model and predict the course of Fabry disease. In order to develop a more extensive, unbiased assessment of the natural history of Fabry disease, Genzyme Corporation has undertaken the conduct of a prospectively defined, epidemiologic study of the natural history of Fabry disease.

This report summarizes the extent of historical data collected in Study Number AGAL-014-01 (Appendix 16.1.1) and updates the data submitted in the two prior interim reports (data collected as of 31 January 2002 and 5 June 2002). Data from all patients that has been collected are included in this report, which provides a comprehensive review on progression of renal disease prior to enzyme replacement therapy (ERT) in this patient population. In addition, this report also addresses progression of cardiovascular disease, cerebrovascular disease, and death rates in this patient population prior to ERT. The information provided in this report encompasses the abstracted medical records from 447 unique patients from 27 sites in five countries (United States, Canada, The Netherlands, Czech Republic, and Denmark). This number meets the goal set by Genzyme to abstract the medical records of 400 patients from all participating sites by June 2002.

8. STUDY OBJECTIVES

The objectives of the study were to:

1. characterize the natural history of Fabry disease
2. estimate the rates of occurrence of renal disease in a population of Fabry patients who have not received enzyme replacement therapy. Progression of cardiac disease, cerebrovascular disease, and/or death was also evaluated
3. provide a historical control for Genzyme r-hαGAL clinical trials
4. characterize the hospitalization of patients with Fabry disease
5. provide support for sample size calculations and conversion from placebo to historical control in Genzyme Study Number AGAL 008-00: Multi-center, Randomized, Double-Blind, Placebo-Controlled Study of the Safety and Efficacy of Recombinant Human α-Galactosidase A (r-hαGAL) on Progression of Renal Disease and Significant Clinical Events in Patients with Fabry Disease.

NOTE: The analyses performed in the efficacy section of this report are based on the AGAL-008-00 Qualified Population to support the conversion of that trial from a double-blind, placebo controlled study, to an open-label, historically controlled study.

9. INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

The basic study approach can be summarized as follows:

1. Use of a historical cohort design that included 27 participating sites with patients who have a current diagnosis of Fabry disease or who had a diagnosis of Fabry disease at the time of death.
2. Sites obtained patients' informed consent and permission to release medical records.
3. Obtaining or accessing patient medical records.
4. Abstraction of medical record information from records into database.
5. Use of standard statistical methods to summarize collected data, estimate incidence rates of events, and estimate survival function.

9.2 Discussion of Study Design, Choice of Control Groups

This was an international, multicenter study of a historical cohort of patients with Fabry disease. Medical record data from this study were used for multiple purposes. For instance, historical control groups were created from the data of patients with Fabry disease who fit the inclusion and exclusion criteria of AGAL-008-00 as well as for other Genzyme studies with r-hαGAL.

Medical records were reviewed from as many patients with Fabry disease as possible from participating study sites. All available, prospectively defined information for these patients was collected.

Four key steps were taken in order to minimize bias in the collection of data from patients' medical records.

- Data were abstracted under the supervision of an independent contract research organization (Abt Associates Clinical Trials (AACT), 55 Wheeler Street, Cambridge, MA 02138) identified as an expert in methodologies of collecting epidemiologic and survey data.
- 51 domestic and international investigator sites were given an opportunity to participate in the study; 27 elected to participate and provided data.
- Records from all eligible patients at participating sites were abstracted.
- Clinically relevant information on Fabry patients was collected with prospectively defined data points.

Additionally, the quality of data was ensured by quality control measures on the part of the Clinical Research Associates (CRA) who abstracted the patients' records. The first two records from each CRA were re-abstracted by an experienced supervisor. Any discrepancies noted by the

supervisor were made apparent to the CRA, and were recorded and initialed on the original abstraction. Subsequently, approximately 10% of each CRA's abstractions were randomly chosen and re-abstracted by supervisors. Further, if patient data were available at more than one site, the data were collected, duplicates were identified, and the patients' records were merged.

The duration of this study was approximately 15 months. There was no restriction on the year of diagnosis of Fabry disease as long as the patient's medical record was available for abstraction. All available, pertinent information for these patients was collected.

9.3 Selection of Study Population

Two steps involved in the selection of the patient population were the selection of eligible sites and the identification and selection of eligible patients within each site. Because patient records may have been transferred between sites, steps were taken by Abt (Associates Clinical Trials) to minimize the chance of duplicating patient data. Abt was required to inform abstractors and supervisors of patient records that have been transferred between sites. The only criterion for the selection of sites was that the site must have reasonable means to facilitate the identification of Fabry patient records.

The goal of the study was to enroll as many patients as possible. The desired minimum enrollment was 400 patients. Overall patient enrollment included hemizygous males (typical), atypical hemizygous males, and heterozygous females, although this information was not distinguished in the database. Medical records from patients with Fabry disease up to the time of enzyme replacement therapy (ERT) were eligible for inclusion.

9.3.1 Inclusion Criteria: AGAL-014-01

For AGAL-014-01, patients were required to meet each of the following criteria to be enrolled in this study:

1. Current diagnosis of Fabry disease, or diagnosis of Fabry disease at time of death.
2. Patient, guardian, or next of kin must provide informed consent, including consent to release medical records

9.3.2 Exclusion Criteria: AGAL-014-01

For AGAL-014-01, patients were excluded from this study if they meet any of the following criteria:

1. Patient had obvious and confounding renal disease (i.e., diabetic nephropathy, SLE, or other well established disorder) at Fabry disease diagnosis.
2. Patient had other major disease (e.g., cancer, HIV/AIDS, significant organic disease) at Fabry disease diagnosis.

Since AGAL-008-00 Qualifiers are identified based on the Phase 4 study (AGAL-008-00) entrance criteria, these criteria have been incorporated into the definition of AGAL-008-00 Qualifiers in this study. A complete list of inclusion/exclusion criteria from the AGAL-008-00 study can be found in the study protocol (Appendix 16.1.1).

9.3.3 Inclusion Criteria: AGAL-008-00 Qualified Population

Patients fulfilling the following criteria taken from the AGAL-008-00 Phase 4 study were eligible for inclusion as part of the AGAL-008-00 Qualified Population to be used as a historical control for the AGAL-008-00 Phase 4 study:

1. The patient must provide written informed consent prior to any study-related procedures being performed.
2. Patients must be ≥ 16 years old.
3. Patients must have a current diagnosis of Fabry disease.
4. Patients may not have received enzyme replacement therapy as treatment for Fabry disease.
5. Patients must have documented plasma α GAL activity of < 1.5 nmol/hr/mL or a documented leukocyte α GAL activity of < 4 nmol/hr/mg.
6. Patients must have one or more of the following:
 - a. serum creatinine measurement ≥ 1.2 and < 3 mg/dL (106.1 and 265.2 μ mol/L)
 - b. estimated creatinine clearance < 80 mL/min (using the Cockcroft-Gault formula (Aronoff, 1995) if the patient's serum creatinine measurements < 1.2 mg/dL.

9.3.4 Exclusion Criteria: AGAL-008-00 Qualified Population

Patients that met the inclusion criteria based on the AGAL-008-00 Phase 4 trial but who fulfilled any of the following exclusion criteria based on AGAL-008-00 were excluded from the AGAL-008-00 Qualified population and from their data being used in the historical control cohort:

1. Patient has undergone or is currently scheduled for kidney transplantation or is currently on dialysis.
2. Patient has acute renal failure.
3. Patient has unconfirmed Fabry disease.
4. Patient has normal α GAL activity.
5. Patient has the following:
 - a. serum creatinine measurement of < 1.2 mg/dL (< 106.1 μ mol/L) (based on Screening period measurements) unless estimated serum creatinine clearance < 80 mL/min, OR
 - b. history of transient ischemic attack (TIA) or ischemic stroke within 3 months of study entry.

6. Patient has received enzyme replacement therapy as treatment for Fabry disease.
7. Patient has diabetes mellitus or presence of confounding renal disease.
8. Patient has current critical coronary artery disease (as documented by a presently unstable angina and/or documented myocardial infarction within 3 months).
9. Patient has congestive heart failure as defined by Class III or Class IV cardiac status as evaluated under the New York Heart Association classification (Vandenbelt, 1996, In: Classical Teachings in Clinical Cardiology).

All renal disease data were collected by each CRA and subsequently assessed by a site investigator who confirmed whether the renal disease was confounding renal disease. This was done since Fabry disease can lead to renal failure and other renal disease manifestations. It was necessary to differentiate between renal disease as a result of Fabry disease and other confounding renal disease from other causes. All data on or after the date of investigator-confirmed confounding renal disease were then excluded.

If HIV diagnosis was on or before the date of Fabry disease diagnosis, then the only data abstracted for that patient were the inclusion and exclusion criteria, medical history, demographics, Fabry disease history, and study site collection.

If a patient had cancer, then the patient's data were still abstracted. All data on or after the diagnosis of cancer were excluded for AGAL-008-00 Qualified patients. If cancer was diagnosed on or before the Qualification Start Date (Section 10.1.1), then the patient did not become an AGAL-008-00 Qualifier.

Likewise, if a patient was diagnosed with diabetes, their data abstracted on or after the date of diagnosis were excluded for AGAL-008-00 Qualified patients. If diabetes was diagnosed on or before the Qualification Start Date, then the patient did not qualify.

9.3.5 Removal of Patients from Therapy or Assessment

Patients' medical record data were not further abstracted once they received any enzyme replacement therapy (ERT). Data collected up to the date of ERT administration were collected and analyzed.

9.3.6 Patient Screening Log

Each site was instructed to maintain a log of each patient's record considered for the study, whether entered into the study or not. All patients identified at the study center were recorded on the screening log by recording the patient's initials and contact dates. If a patient was not consented, the reason was recorded on the screening log. Date of birth was added to the screening log after consent was given so that duplicate patients could be identified.

To date, a total of 742 patients have been recorded on screening logs, though a small number of screening logs are still outstanding and have not been forwarded by the sites. Table 9-1 summarizes the screening logs received to date and categorizes the reason a patient was not included in the historical database. Of the 447 patients that agreed to participate in the study, a total of 431/447 (96%) of the patients noted on the screening logs have been received by Genzyme from the sites. Of the 742 patients noted on the screening logs received, 311 (42%) did not participate in the study. Among these patients “no response” was the most common reason for non-participation (Table 9-1).

Table 9-1: Summary of Screening Logs

Reason not Included in the Study	Frequency (%)	
No Response	264	(36%)
No / Not Interested	24	(3%)
No / Patient Deceased	8	(1%)
No / Wrong Address	12	(2%)
No / Negative for Fabry	1	(0%)
No / Participating in another Study	2	(0%)
Yes*	431	(58%)

* The total number of subjects in the AGAL-014-01 database is 447, at present all of the screening logs have not been received.

9.4 Treatments

Not Applicable

9.4.1 Treatments Administered

Not Applicable

9.4.2 Identity of Investigational Product

Not Applicable

9.4.3 Method of Assigning Patients to Treatment Groups

Not Applicable

9.4.4 Selection of Doses in the Study

Not Applicable

9.4.5 Selection and Timing of Dose for Each Patient

Not Applicable

9.4.6 Blinding

Not Applicable

9.4.7 Prior and Concomitant Therapy

Information on patients treated concomitantly with ACE inhibitors/Angiotensin Converting Enzyme Receptor Blockers (ARBs) was collected (see Section 11.4.3 and Section 14.4).

9.4.8 Treatment Compliance

Not Applicable

9.5 Efficacy and Safety Variables

Prospectively defined study measurements included progression of renal disease, cardiac disease, cerebrovascular disease, and/or death. Note that summary tables and listings of renal, cardiac, cerebrovascular, and death data are presented in Appendix 16.2.9 for review.

When compiling historical data, it is important to understand that certain types of data may be more accurately obtained than others. For example, when assessing renal function and changes in renal function over time, one can generally rely on measurements of serum creatinine and/or estimated GFR. This is a well-accepted and standardized test available in clinical laboratories throughout the world. Conversely, accurately assessing the incidence of cardiac events (such as myocardial infarctions, worsening angina, worsening congestive heart failure, and clinically important arrhythmias) and cerebrovascular events (such as strokes and transient ischemic attacks) is much more subjective.

These latter types of assessments rely heavily on a physician's clinical assessment of a variety of different types of data including medical history, physical examination, various types of laboratory data including several different types of enzyme assays and electrocardiograms, and several types of radiologic assessments. Such clinical assessments always have a significant subjective component and are certainly not nearly as objective as the measurement of a standard lab test such as a serum creatinine, which is more likely to appear in a standard lab report. The criteria for evaluation of cardiac and cerebrovascular events are detailed and very stringent; often these details are not found in a patient's medical record. Hence, the historical assessment of cardiac and cerebrovascular event rates is likely to be underestimated. In addition, non-study physicians may have seen patients. While attempts were made to obtain a patient's medical records from all physicians seen by the patient, this was not always possible. Fabry disease impacts many different body systems, and often patients are seen by many different physicians in different specialties, some of whom were not study physicians. Therefore, patient's medical records may not have been updated completely with their entire medical history if more than one physician compiled them. For all of these reasons, while we have analyzed the incidence of cardiac and cerebrovascular events occurring in the Fabry historical data base, we are relying on

the much more accurate historical assessments of renal function in providing the historical control for AGAL-008-00.

9.5.1 Renal Disease

Progression of chronic and/or end stage renal disease was met based on one of the following conditions:

1. Progression of chronic renal disease was met by an increase of at least 33% in serum creatinine levels (from first measurement). Progression of chronic renal disease based on an increase of 50% in serum creatinine levels (from first measurement) was also reported.
2. Progression to end stage renal disease (not acute renal failure) as defined by a requirement for intervention therapy such as the need for chronic dialysis continuing for > 40 days or renal transplantation.

9.5.2 Cardiac Disease

Progression of cardiac disease met by Myocardial Infarction (MI), Change in Cardiac Status, Arrhythmia, Angina, or Cardiac Failure is presented.

Myocardial Infarction:

Definite ECG change, Probable ECG change with symptoms and abnormal enzymes and Death by MI.

Other sub-events mentioned below are presented directly from entries on CRFs.

- Change in Cardiac Status: PTCA, IABP, CABG, Valve replacement (Ischemic), Cardiac hospitalization
- Arrhythmia: Symptoms of arrhythmia, Anti-arrhythmic medication, DC-cardioversion, Pacemaker, Defibrillator
- Angina: Rest angina, Increasing angina, Change in resting EKG with angina pain, New onset angina
- Cardiac Failure: Physical findings on exam, Exercise intolerance, Cardiac imaging, IV medications given

9.5.3 Cerebrovascular Disease

Cerebrovascular disease defined by stroke and TIA are presented.

Types of events listed below are presented directly from entries on CRFs.

- TIA: R Carotid hemispheric, L Carotid hemispheric, R Amaurosis fugax, L Amaurosis fugax, Vertebrobasilar, Uncertain
- Stroke: Hemorrhage, Type: Subarachnoid, Labar, Non Labar (Basal Ganglia, Brain Stem-diencephalon, Cerebellum, Other), Infarct, Type: Embolic, Thrombotic, Uncertain, Vessel size: Small (lacunar), Large, Side: Right or Left, Location: Anterior Cerebrum, Middle Cerebrum, Posterior Cerebrum, Vertebrobasilar

9.5.4 Death

Death due to any cause.

9.5.5 Efficacy and Safety Measurements Assessed and Flow Chart

Not Applicable

9.5.6 Appropriateness of Measurements

Not Applicable

9.5.7 Primary Efficacy Variable(s)

Not Applicable

9.5.8 Drug Concentration Measurements

Not Applicable

9.6 Data Quality Assurance

The medical records were to be abstracted and CRFs were to be reviewed and then returned to Genzyme for data management and analysis. Investigators were required to complete and sign the CRF that determined if renal disease was a result of Fabry disease or other confounding renal disease. If necessary, the study site was to be contacted for corrections and/or clarifications. All data was to be entered into a study database for analysis and reporting. Validation programs were to be run on the data for purposes of quality assurance (QA). Upon completion of data entry, the database was to receive a QA check to ensure acceptable accuracy and completeness.

9.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

All data maintained by Genzyme BioMedical Operations will be entered and managed in an Oracle database using Domain Solutions/Clintrial on a VAX/VMS computer. Statistical analyses

were performed using the SAS Software package in a validated environment. For statistical modeling, the S-PLUS and SAS software packages were employed.

9.7.1 Statistical and Analytical Plans

Data are presented through data listings or tables. For tables, continuous outcomes are summarized with n's, means, standard deviations, medians, minimums, and maximums. Categorical variables are summarized with frequency counts and percentages. Graphical displays are presented as appropriate.

For estimating the renal event rate the simplest method was employed, specifically empirical estimate based on tabulating the proportion of patients who were observed to have the event within a certain time period.

For comparison to the AGAL-008-00 trial, more precise and robust estimates of the incidence rate were obtained by modeling the underlying patient-specific trends in serum creatinine over the full duration of follow-up.

Time to occurrence was estimated for death using the Kaplan Meier methods. The survival function was graphically displayed.

9.7.2 Efficacy Endpoint Data

Each type of event (Renal, Cardiac, CVD, Death, Any Event) described in Section 9.5 was tabulated along with sub-events or type of event.

9.7.2.1 Renal Endpoint Data

9.7.2.1.1 Progression of Renal Disease: Dialysis and Transplantation

Acute Dialysis (length \leq 40 days), Chronic Dialysis (length $>$ 40 days), length of dialysis, and Transplantation are presented.

9.7.2.1.2 Progression of Renal Disease for the AGAL-008-00 Qualified Population: Serum Creatinine

Renal event rates are estimated using linear random effects models based on the underlying patient-specific trend in serum creatinine over the full duration of follow-up. The empirical estimate based on tabulating the proportion of patients who were observed to have had a 33% increase in serum creatinine, or who required dialysis (length $>$ 40 days), or kidney transplantation within the first 2 years from the Qualification Start Date (Section 10.1.1) was biased as some patients had less than 2 years follow-up. This was because patients who had been followed for fewer than 2 years had a lower probability of event, and inclusion of these patients in the empirical estimation of event rate was biased (under-estimated). In order to have more precise and robust estimates of the event rate, the linear random effects model was employed.

The model demonstrated that log-serum creatinine depended on the value at entry (intercept) and time since entry. The linear rate of change in log-serum creatinine over time (slope) depended on the patient. The model was fit by the method of restricted maximum likelihood, and the underlying patient-specific trend is determined from the empirical Bayes estimates of the random intercept and slope. On the log scale, the individual slope is directly related to the expected percent change in serum creatinine.

A linear random effects model was fit to the data of the AGAL-008-00 Qualifiers, using the method of restricted maximum likelihood estimation. Individual patient slopes are estimated using empirical Bayes estimation (Laird and Ware 1982). The 80%, 84%, 90%, and 95% confidence intervals on the event rate were estimated.

The primary model to assess the estimated renal event rate was a linear trend random effects model (see SAP) for serum creatinine, with a 33% 2-year criteria and a 50% 3-year criteria. The following supplementary analyses were also performed to assess the robustness of the renal event rate:

- Analyzing the data and omitting patients with limited observations (patients with ≥ 2 observations, patients with ≥ 3 observations)
- Adjusting for covariates (age, gender, weight, blood type, and plasma α -GAL level)
- Empirical estimates of renal event rate
- Inclusion of a quadratic term
- Investigation of 1/serum creatinine transformation
- By-subject linear regression
- Analysis of estimated GFR.

In addition, individual serum creatinine measurements were plotted over time along with usage of ACE Inhibitors/ARBs and blood pressure measurements. Also, serum creatinine measurements at Qualification Start Date were presented descriptively for AGAL-008-00 Qualifiers.

9.7.2.1.3 Estimated Glomerular Filtration Rate (GFR) using the MDRD Equation for the AGAL-008-00 Qualified Population

The estimated Glomerular Filtration Rate (GFR) is based on the MDRD formula that takes the patient's serum creatinine values, age, gender, and race into consideration (see Appendix 16.1.9). The estimated GFR was presented at 2 years, 3 years, 4 years, and 5 years from Qualification Start Date. For each time period, the last GFR estimated from each patient was summarized.

Using the same linear random effects modeling methods, renal event rates were estimated by using a patient's log transformed GFR values as opposed to log transformed serum creatinine values. This analysis was performed with the following exceptions:

- Log-transformed GFR was the response variable as opposed to log transformed serum creatinine
- Increases in serum creatinine rates of 50% over 3-years and 33% over 2-years were used to define slope cut-points of 0.135 and 0.143 respectively for the mixed model analysis of log-serum creatinine. Corresponding slope cut-points for estimated GFR based on the 50% increase over 3-years and 33% over 2-years criteria using the MDRD estimated GFR formula were calculated. The patients slope cut-points for the estimated GFR were - 0.161 (corresponding to the 50% 3-year criteria) and - 0.170 (corresponding to 33% 2-year criteria). For the mixed model analysis of the natural log of estimated GFR, patients with an estimated slope equal to or less than these cut-points were defined as having had estimated renal event.
- For the supplemental analyses incorporating covariates into the model: age, gender, weight, blood type, and plasma α-GAL level were used.

9.7.2.2 Cardiac Endpoint Data

Cardiac events described in Section 9.5.2 were tabulated along with sub-events or type of event.

9.7.2.3 Cerebrovascular Endpoint Data

Cardiac events described in Section 9.5.3 were tabulated along with sub-events or type of event.

9.7.2.4 Death Endpoint: Kaplan-Meier Estimate of Mortality Rate

A figure presenting the Kaplan-Meier estimates of mortality starting from date of birth was produced for all patients. If patients did not have a record of death, the most current date in a patient's entire records collected in this study was used as a censoring point (See Appendix 16.1.9).

9.7.2.5 Time to Events

For AGAL-008 Qualifiers, the following time will be presented descriptively; Years from Qualified Start Date to:

- Renal Event [33% criteria]: earliest date of either a 33% increase in serum creatinine, chronic dialysis (length > 40 days), or kidney transplantation
- Renal Event [50% criteria]: earliest date of either a 50% increase in serum creatinine, chronic dialysis (length > 40 days), or kidney transplantation
- Cardiac Event
- Cerebrovascular Event
- Death

9.7.3 Determination of Sample Size

Not applicable for AGAL-014-01 study. Refer to Section 11.5.9 for Sample Size Justification for Study Number AGAL-008-00.

9.7.4 Missing or Invalid Data

Since this is a retrospective study and time (date) is a very sensitive factor for the analyses, stringent rules were set and applied to these historical data in order to utilize them with most meaningful way (see Appendix 16.1.9).

9.8 Changes in the Conduct of the Study or Planned Analyses

Based upon communications with the FDA, the following additional analyses were conducted to support the methodology and results of the renal event rate estimation analyses:

- Justification of the natural logarithm transformation for serum creatinine
- A review of the distribution of the subject slopes and intercepts
- An analysis of renal event rates based upon applying empirical estimation methods
- Additional covariate analyses
- Justification of 84% confidence intervals for the event rates.

10. STUDY PATIENTS

10.1 Disposition of Patients

10.1.1 Establishing the “Qualified” Population

The database created by AGAL-014-01 was based on collection of data from all types of patients with Fabry disease. As other studies on Fabry disease are conducted, patient records in the AGAL-014-01 historical database will be matched to the other studies’ inclusion and exclusion criteria to establish a subset of records to serve as a reasonable comparator.

The medical records of patients from AGAL-014-01 were abstracted to document several relevant facts of the patients’ medical histories. Once a database of medical record abstractions was established, patients from this database were compared to AGAL-008-00 inclusion/exclusion criteria to establish a “Qualified” population of patients. The process for identifying each qualified patient was to:

1. Identify each patient who met the inclusion criteria of AGAL-008-00.
2. Establish a patient-specific date (“Qualification Start Date”) against which the date of any disqualifying event for that patient would be measured. The Qualification Start Date was determined as the earliest date on which the patient satisfied all inclusion criteria of Study No. AGAL-008-00.
3. Exclude any patient who had an exclusion event (defined by AGAL-008-00) prior to or on the Qualification Start Date (see Table 10-2).

This process was followed because of the differences between a traditional, prospective clinical study and a study involving the historical review of medical records. In a traditional, prospective clinical study, patients would be physically screened in order to determine eligibility for enrollment. In that situation, a specific time period is created based on the screening process (e.g., standard lab draws, inclusion criteria), and potential exclusion events discovered in the screening process that would pre-empt the patient from study enrollment. In the case of an historical review of patients’ medical records, in which a patient is not physically screened, a screening period must be determined in order to establish a date against which to measure the dates of exclusion events. For example, the preliminary review of a patient’s medical history may indicate serum creatinine levels that would permit study enrollment. However, if upon further review of the medical history, an exclusion event was found, that may lead to the patient’s exclusion from the study. However, it is important to note that the relationship of the dates of the serum creatinine levels and the exclusion event must be known before the patient can be excluded. Therefore, a patient-specific “Qualification Start Date” was established, and this date was used as the point in time against which exclusion events would be measured. Just as in

a traditional, prospective clinical study, if an exclusion event occurred prior to or on the Qualification Start Date, the patient would be excluded.

From this process, two populations were identified in the study: a “Qualified” population of 104 patients that met all inclusion and had no exclusion criteria of AGAL-008-00, and the total population of 447 patients that represented the abstracted medical records of patients with Fabry disease from all of the participating sites. (Table 10-2)

Table 10-1: Summary of Inclusion/Exclusion of Patients

Patients	n (%)
Total Number of Unique Patients	447
Patients Meeting All Inclusion Criteria	116 (26)
Patients Who Met All Inclusion Criteria and Had Exclusion Events	12 (3)
Patients Meeting All Inclusion Criteria and Having No Exclusion Events	104 (23)
Reference: Table 14.1-1	

10.1.2 Detailed Patient Disposition

Table 10-2 summarizes patient disposition based on AGAL-008-00 inclusion/exclusion criteria. For the Qualified population, the range of each patient’s data available for evaluation was based on each patient’s Qualification Start Date (refer to Section 10.1.1) and the “Stop Date” if available. The Stop Date was defined for some patients as the date on which a patient began receiving enzyme replacement therapy for Fabry disease or developed confounding renal disease. For apparent reasons, it would not be appropriate to evaluate data collected past the date of either ERT or development of confounding renal disease. A Stop Date was defined for all patients whether or not they qualified, and data on or after this date were excluded. For example, some exclusion events had a 3-month window (e.g. MI and TIA) such that if one of these events occurred within 3 months of the Qualification Start Date, then the patient would not qualify. In this situation a later Qualification Start Date was determined such that an exclusion event would no longer apply (i.e., there was a greater than 3 month time span from the time of the event to the new Qualification Start Date).

Table 10-3 provides a summary of the duration that AGAL-008-00 Qualified patients were included in the study. This was based on the amount of time elapsed between their Qualification Start Date and Stop Date, and is the time window in which data was analyzed for the purpose of serving as a historical control for the AGAL-008-00 study. The mean time of study duration for the AGAL-008-00 Qualified patients was 3.6 years (SD=4.4 years). For 39/104 (37%) of the AGAL-008-00 Qualified patients, study duration was < 1 year. Thirty-five of 104 (34%) AGAL-008-00 Qualified patients had study durations of 1 to < 5 years, and 30/104 (29%) patients had study durations of > 5 years.

Table 10-2: Summary of Patient Disposition Based on AGAL-008-00 Inclusion/Exclusion Criteria

Criteria	n (%)*
Total number of patients	447 (100)
Inclusion Criteria that Define Qualification Start Date	
Age of 16 Years or Older at Lab Draw Date	387 (87)
Serum Creatinine ≥ 1.2 and < 3 mg/dL	141 (32)
Serum Creatinine < 1.2 and Estimated Creatinine Clearance < 80 mL/min	65 (15)
Other Inclusion Criteria	
Plasma αGAL Activity < 1.5 nmol/hr/mL	120 (27)
Leukocyte αGAL Activity < 4 nmol/hr/mg	126 (28)
Exclusion Events	
Transplant At or Before Qualification Start Date	20 (4)
Diabetes At or Before Qualification Start Date	2 (0)**
Dialysis At Start of Qualification Start Date	0
Malignant Cancer At or Before Qualification Start Date	5 (1)
TIA Within 3 Months of Qualification Start Date	1 (0)**
MI Within 3 Months of Qualification Start Date	0
Type III Or IV Cardiac Failure At or Before Qualification Start Date	4 (1)
Defines Qualification Stop Date	
On Enzyme Replacement Therapy	119 (27)
Confounding Renal Disease	7 (2)
Reference: Table 14.1-1 * Rows are not mutually exclusive; patients may have experienced more than one exclusion criteria. **Because of rounding 2 or fewer patients are represented as 0% of the population (actual = 0.4% or less).	

Table 10-3: Overall Study Duration and Study Duration by Time Groups for AGAL-008-00 Qualified Patients

Study Duration (yrs.)	
Mean ± SD	3.6 ± 4.4
Median	2.1
Min., Max.	0, 27
Total Patients	104
Duration by Time Groups	
	n (%)
0 - <1 yr	39/104 (37)
1- <2 yrs	11/104 (11)
2- <3 yrs	12/104 (11)
3- <4 yrs	8/104 (8)
4- <5 yrs	4/104 (4)
≥5 yrs	30/104 (29)
Reference: Table 14.1-9	
Note: Study duration is calculated from the Qualification Start Date until the Stop Date or the last date in the patient's entire record of data collected in this study. The Stop Date is the earliest date on which a patient began receiving enzyme replacement therapy (ERT) for Fabry disease or developed confounding renal disease confirmed by the investigator; if a patient did not have ERT or confounding renal disease, no Stop Date will exist.	

10.2 Protocol Deviations

There were no deviations from the protocol.

10.2.1 Deviations from Planned Analyses

Based on conversations with FDA surrounding the estimation of the renal event rate for the AGAL-008-00 Qualified Population, the following supplementary analyses were performed:

- Renal event rates were estimated based on the random effects model being fit to qualifying patients who had ≥ 2 observations and to qualifying patients who had ≥ 3 observations.
- The distribution of the estimated patient slopes and intercepts from the random effects model were reviewed.
- The distribution of the estimated patient slopes was reviewed by means of stratifying the estimated slopes based on subject covariates.
- Renal event rates were estimated based upon applying empirical methods.
- Renal event rates were estimated based on the random effects model with covariate terms included in the model.
- Renal event rates were estimated based on analysis of the data by means of individual patient regressions.
- A likelihood based approach to the renal event estimate.
- Exclusionary subset analyses.
- Analysis of 1/serum creatinine.

11. EFFICACY EVALUATION

11.1 Data Sets Analyzed

11.2 Demographic and Other Baseline Characteristics

Table 11-1 summarizes demographic data of the entire historical population as of the time of the patient’s diagnosis with Fabry disease, including weight, height, gender, and ethnicity.

Table 11-1: Summary of Demographics of the Historical Patient Population at Fabry Disease Diagnosis

Parameter	Statistic	All Patients n = 447 At Fabry Diagnosis
Age (yr)	n	404
	Mean	26.0
	Median	25.0
	Std. Dev.	15.31
	Min., Max.	0, 76
Weight (kg)	n	143
	Mean	65.3
	Median	68.9
	Std. Dev.	19.97
	Min., Max.	4, 131
Height (cm)	n	124
	Mean	165.3
	Median	169.0
	Std. Dev.	19.66
	Min., Max.	46,189
Gender		
Male	n (%)	279 (62)
Female	n (%)	168 (38)
Ethnicity		
Caucasian	n (%)	382 (85)
Black	n (%)	8 (2)
Hispanic	n (%)	25 (6)
Asian	n (%)	1 (0)
Other	n (%)	26 (6)
Reference: Table 14.1-3		

Table 11-2 summarizes the available demographic data of the Historical patient population at the Qualifying Start Date compared to the Phase 4 Study (AGAL-008-00) study population at Baseline.

For patients in the AGAL-008-00 Qualified historical population (n = 104), the mean age at the Qualification Start Date was 37.7 years (SD = 10.27), and the median age among these same patients was 37.2 years. The mean serum creatinine level at the Qualification Start Date was 1.5 mg/dL. For patients randomized in the Phase 4 study (AGAL-008-00) (n = 61), the mean age at Baseline was 45.8 years (SD = 9.86), and the median age among these same patients was 44.8 years. The mean serum creatinine level at Baseline of the Phase 4 study (AGAL-008-00) was 1.7 mg/dL.

Table 11-2: Summary of Demographics of the Qualified Historical Patient Population at Qualification Start Date Compared to the Phase 4 Study (AGAL-008-00) Population at Baseline

Parameter	Statistic	Historical Patients Who Are AGAL-008-00 Qualifiers n = 104	Patients Randomized in AGAL-008-00 n = 61*
		At Qualification Start Date	At Baseline
Age (yr)	n	104	61
	Mean ± SD	37.7 ± 10.27	45.8 ± 9.86
	Median	37.2	44.8
	Min., Max.	16, 68	20.8, 68.3
Serum Creatinine (mg/dL)	n	104	61
	Mean ± SD	1.49 ± 0.466	1.7 ± 0.54
	Median	1.3	1.5
	Min., Max.	0.80, 2.90	0.9, 2.9
GFR (mL/min/1.73m ²)	n	104	61
	Mean ± SD	61.85 ± 18.67	52.4 ± 17.09
	Median	63.75	52.8
	Min., Max.	25.12, 127.67	25.0, 97.3
Weight (kg)	n	67	60
	Mean ± SD	70.3 ± 12.42	71.2 ± 11.85
	Median	70.5	70.2
	Min., Max.	32, 98	50.0, 103.4
Height (cm)	n	57	59
	Mean ± SD	174.1 ± 10.26	171.9 ± 16.12
	Median	175.3	172.7
	Min., Max.	124, 191	68, 195
Gender			
Male	n (%)	98 (94)	54 (89)
Female	n (%)	6 (6)	7 (11)
Ethnicity			
Caucasian	n (%)	88 (85)	55 (90)
Black	n (%)	3 (3)	1 (2)
Hispanic	n (%)	8 (8)	3 (5)
Asian	n (%)	1 (1)	1 (2)
Other	n (%)	3 (3)	1 (2)

Reference: Table 14.1-3 (AGAL-014-01), Table 14.1-3 (AGAL-008-00), Table 14.2.1-3 (AGAL-008-00), and Table 14.8-1 (AGAL-014-01) *These descriptive statistics are based on the preliminary data as the AGAL-008-00 study is still ongoing.
 Note: GFR estimated using the MDRD equation.

Table 11-3 summarizes additional Fabry disease history data of the AGAL-008-00 Qualified population and the total population, presented side-by-side for comparison.

The remaining demographic data presented in Table 11-3 include blood type, family members diagnosed with Fabry disease, plasma αGAL activity (nmol/hr/mL), and leukocyte αGAL activity (nmol/hr/mg).

Table 11-3: Interim Review: Summary of Fabry Disease History

Parameter	Statistic	Historical Patients Who Are AGAL-008-00 Qualifiers n = 104	All Patients n = 447
Blood Type			
{A+, A-, O+, O-}	n (%)	56 (54)	225 (50)
{B+, B-, AB+, AB-}	n (%)	11 (11)	24 (5)
Unknown	n (%)	37 (36)	198 (44)
Family Members Diagnosed with Fabry			
Yes	n (%)	84 (81)	388 (87)
Males	n	67	343
Females	n	73	327
No	n (%)	13 (13)	36 (8)
Unknown	n (%)	7 (7)	23 (5)
Plasma αGAL Activity (nmol/hr/mL)	n	56	184
	Mean	0.71	2.85
	Median	0.40	1.49
	Std. Dev.	0.604	4.612
	Min., Max.	0.00, 1.49	0.00, 40.30
Leukocyte αGAL Activity (nmol/hr/mg)	n	48	203
	Mean	1.29	12.68
	Median	0.95	2.00
	Std. Dev.	1.198	22.766
	Min., Max.	0.00, 3.90	0.00, 135.60
Reference: Table 14.1-4			
Note: Because of rounding, all percentages in a category may not equal 100.			

Table 11-4 summarizes general medical history data for the AGAL-008-00 Qualified population and the total population, presented side-by-side for comparison. It is important to note that the data in Table 11-4 was taken directly from the case report forms and does not take into account the Qualification Start Date.

Table 11-4: Summary of Medical History

Parameter	Response	Statistic	AGAL-008-00 Qualifiers n = 104	All Patients n = 447
Diagnosis of Cancer	Yes	n (%)	6 (6)	23 (5)
	No	n (%)	97 (93)	423 (95)
HIV Positive	Yes	n (%)	0	0
	No	n (%)	104 (100)	446 (100)
Diabetic	Yes	n (%)	1 (1)	13 (3)
	No	n (%)	103 (99)	434 (97)
Renal Disease¹	Yes	n (%)	48 (46)	134 (30)
	No	n (%)	56 (54)	313 (70)
Clinical Trial of Enzyme Replacement Therapy	Yes	n (%)	67 (64)	119 (27)
	No	n (%)	37 (36)	327 (73)
Cirrhosis	Yes	n (%)	1 (1)	1 (0)
	No	n (%)	101 (97)	440 (98)
Chronic Hepatitis Type B	Yes	n (%)	0	0
	No	n (%)	0	0
Chronic Hepatitis Type C	Yes	n (%)	1 (1)	3 (1)
	No	n (%)	0	0
Hypertension with Meds	Yes	n (%)	33 (32)	118 (26)
	No	n (%)	69 (66)	324 (72)
Intracardiac/Arteriovenous Shunt/Fistula	Yes	n (%)	5 (5)	30 (7)
	No	n (%)	96 (92)	407 (91)
Hyperthyroidism	Yes	n (%)	3 (3)	9 (2)
	No	n (%)	99 (95)	431 (96)
Hypercholesterolemia	Yes	n (%)	18 (17)	61 (14)
	No	n (%)	83 (80)	377 (84)

Reference: Table 14.1-5
 1 Renal disease included chronic renal insufficiency, chronic renal failure, and proteinuria, as documented in the patient's medical record.
 Note: The data presented in this table is from the CRF (not manipulated) and does not take into account the date of AGAL- 008- 00 qualification.
 Note: Because of rounding, all percentages in a category may not equal 100.
 Note: Values for n may change based on the availability of the data.

Renal disease was recorded by the abstractor if the patient's medical record stated that the patient had any renal disease (due to Fabry disease or other cause). If a patient had a diagnosis of "renal disease," then the investigator was asked to fill out a form that requested clarification on whether this was a "confounding renal disease." If the patient had a confounding, renal disease then the data were analyzed only until the date of the diagnosis of the confounding renal disease.

For patients in the Qualified population (n = 104), 48/104 (46%) patients were reported as having renal disease (e.g., chronic renal insufficiency, chronic renal failure, proteinuria) and 56/104 (54%) patients were reported as not having renal disease. One patient from the qualified population was reported to have confounding renal disease. Treatment of hypertension with medication was reported for 33/104 (32%) patients.

For patients in the total population (n = 447), 134/447 (30%) patients were reported as having renal disease (e.g., chronic renal insufficiency, chronic renal failure, proteinuria), and 313/447 (70%) patients were reported as not having renal disease. Thirteen of 447 (3%) patients were reported as being diabetic. Treatment of hypertension with medication was reported for 118/447 (26%) patients who had such available data.

The remaining medical history data presented in Table 11-4 include diagnosis of cancer, diagnosis of diabetes, HIV status, enzyme replacement status, presence of cirrhosis, status and strain of hepatitis infection, presence of intracardiac/arteriovenous shunt/fistula, status of hyperthyroidism, and status of hypercholesterolemia.

11.3 Measurements of Treatment Compliance

Not Applicable

11.4 Efficacy Results and Tabulations of Individual Patient Data

11.4.1 Summary of Events

Table 11-5 is a summary of all renal, cardiac, cerebrovascular events and death, representing progression of Fabry disease in the AGAL-008-00 Qualified patient population from the historical database. The overall number of cardiac, cerebrovascular, and renal events are summarized as well as major sub-categories from each of these overall categories from the time of the patient's Qualification Start date to time-points ranging from 2 to 5 years. The total number of events throughout the qualification period (all available years) is also presented.

As shown in Table 11-5, the number majority of the events that are indicators of Fabry disease progression in each overall category occurred within 2 to 4 years after the patient's qualification start date with the exception of death. The overall number of events increased progressively over the time periods evaluated.

Table 11-5: Summary of All Events

Event	2 years	3 years	4 years	5 years	All Available Years
Qualified Patients = 104	Number of Patients at Each Time Point				
Cardiac	29	36	39	43	49
MI	0	0	0	2	2
Change in Cardiac Status	1	3	3	6	7
Arrhythmia	24	29	32	36	42
Angina	4	8	10	10	14
Cardiac Failure	1	2	2	2	4
Cerebrovascular	3	5	6	6	10
TIA	1	1	1	1	2
Stroke	2	4	5	5	8
Renal (33% criteria)	13	16	17	19	26
33% Increase Serum Creatinine	12	15	16	18	25
Chronic Dialysis (> 40 days)	3	3	6	6	7
Transplantation	0	1	1	1	1
Renal (50% criteria)	11	13	16	17	26
50% Increase Serum Creatinine	10	12	15	16	25
Chronic Dialysis (> 40 days)	3	3	6	6	7
Transplantation	0	1	1	1	1
Death	0	0	2	2	7
Any Event	41	48	52	55	61
Reference: Table 14.7-1					

11.4.2 Hospitalization of Patients in the Historical Database

Information on hospitalizations from patients in the historical database is included in Appendix 16.2.9. The hospitalization data is compiled for both AGAL-008-00 Qualified patients as well as the total group of patients (N=447) and includes dates of admission, primary diagnoses, and both primary and secondary diagnoses at the time of discharge. A total of 96/104 (92%) of AGAL-008-00 Qualified patients were hospitalized at least once. A total of 363/447 (81%) of the entire patient population were hospitalized at least once.

11.4.3 Cardiac Events

Because of the difficulties inherent in collecting historical data based on subjective parameters (see Section 9.5) it was not possible to perform formal statistical modeling to predict event rates for cardiac disease progression. Because of subjectivity in collecting cardiac data, the number of actual events reported is low and not robust enough to allow for the type of modeling that was performed for estimation of renal event rates.

A total of 49/104 (47%) patients in the AGAL-008-00 Qualified population experienced a cardiac event as defined in Section 9.5.2. Table 11-6 presents both the mean time (in years) from the patient's Qualification Start date to the time of occurrence of a cardiac event and the patient's age at the time of occurrence of the event. A detailed listing of the types of cardiac events that occurred is presented in Table 14.5.

Table 11-6: Mean Time (in Years) to Cardiac Event Occurrence in AGAL-008-00 Qualified Patients

	# Years from AGAL-008-00 Qualification Start to Event	Age at Event
# Patients	49	49
Mean ± SD (yrs)	2.4 ± 2.66	42.7 ± 8.29
Median (yrs)	1.4	42.1
Min., Max. (yrs)	0.0, 9.3	21.1, 63.7
Reference: Table 14.7-2; Table 14.7-3		

Additional data collected for the AGAL-008-00 Qualified population included the number of patients that were treated for hypertension with ACE inhibitors and/or ARBs (ACE Receptor Blockers) medications (see Table 14.10). A total of 33/104 (32%) of patients were treated with ACE inhibitors and 5/104 (5%) were treated with ARBs. Individual serum creatinine measurements were plotted over time along with usage of ACE Inhibitors/ARBs-ACE Receptor Blockers and blood pressure measurements (see Section 14.4).

11.4.4 Cerebrovascular Events

Because of the subjective nature of collecting historical data for cerebrovascular events, it was not possible to perform formal statistical modeling to predict event rates for cerebrovascular disease progression (see Sections 9.5 and 11.4.2).

A total of 10/104 (10%) patients in the AGAL-008-00 Qualified population experienced a cerebrovascular event as defined in Section 9.5.3. Table 11-7 presents both the mean time (in years) from the patient's Qualification Start date to the time of occurrence of a cerebrovascular event and the patient's age at the time of occurrence of the event. A detailed listing of the types of cerebrovascular events that occurred is presented in Table 14.6.

Table 11-7: Mean Time (in Years) to Cerebrovascular Event Occurrence in AGAL-008-00 Qualified Patients

	# Years from Qualification Start to Event	Age at Event
# Patients	10	10
Mean ± SD (yrs)	5.9 ± 7.92	48.4 ± 8.53
Median (yrs)	3.0	47.3
Min., Max. (yrs)	0.0, 26.8	36.7, 61.0
Reference: Table 14.7-2; Table 14.7-3		

11.4.5 Death

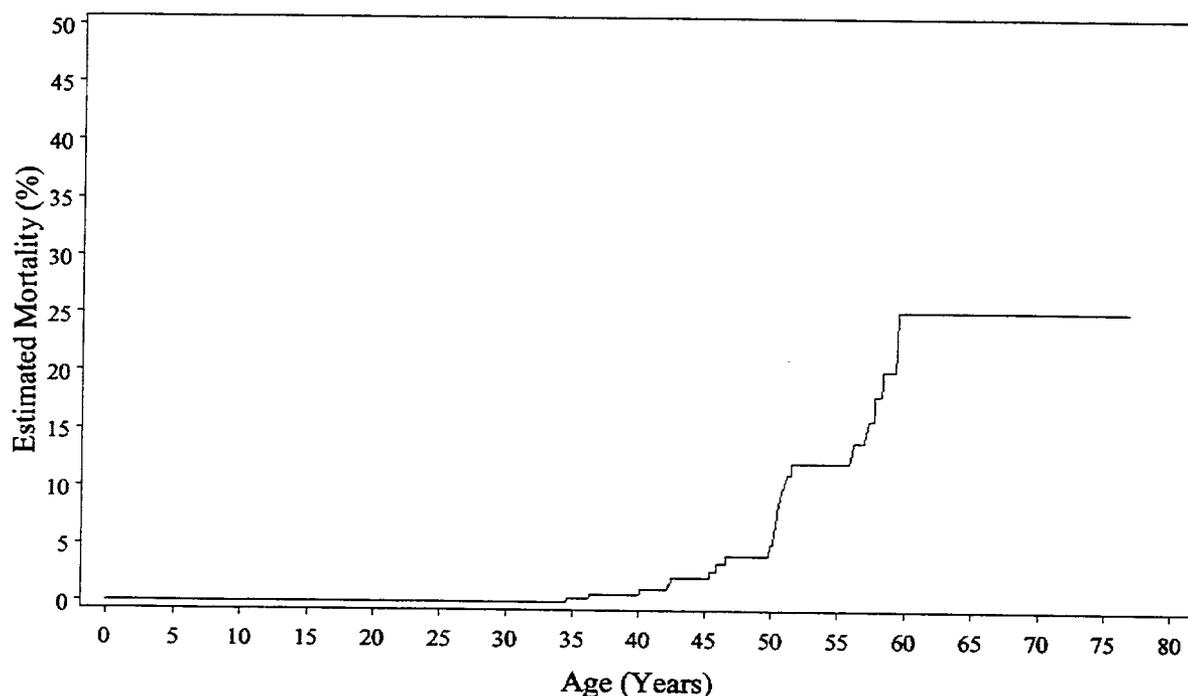
Seven of 104 (7%) patients meeting the AGAL-008-00 inclusion/exclusion criteria died. The mean time (in years) from the patient’s Qualification Start date to the time of death and the patient’s age at the time death are presented in Table 11-8. Two of the AGAL-008-00 qualified patients died as a result of end stage renal disease related to Fabry disease and one patient died as a result of acute leukemia. The cause of death was unknown for three of seven patients and not recorded for one patient.

Table 11-8: Mean Time (in Years) To Death in AGAL-008-00 Qualified Patients

	# Years from Qualification Start to Death	Age at Time of Death
# Patients	7	7
Mean ± SD (yrs)	7.2 ± 3.00	52.1 ± 6.86
Median (yrs)	6.8	50.4
Min., Max. (yrs)	3.6, 12.1	42.4, 59.4
Reference: Table 14.7-2; Table 14.7-3		

A total of 22/447 (5%) died in the study population as a whole. Figure 11-1 presents the estimated mortality rate as determined by Kaplan-Meier life table analysis. This analysis is based on the total number of patients (N=447) collected in the database, not just AGAL-008-00 qualifiers, and includes the patient’s entire medical history that was available for abstraction. As shown in Figure 11-1, based on the medical records collected, the estimated mortality rate at 60 years of age is approximately 25%. However, only a limited number of sites obtained authorization from next of kin for medical records of patients that had died, therefore this is likely an underestimation of the mortality rate. In addition, patients that did not participate in the study due to “no response” on the Screening Logs may be deceased and also contribute to an underestimation of mortality.

Figure 11-1: Kaplan-Meier Estimated Mortality Rate for Entire Study Population



11.4.6 Renal Events

Table 11-9 is a summary of the number of patients that experienced actual renal events that occurred in the AGAL-008-00 Qualified population based on both the 33% and 50% criteria described in Section 9.5.1. A total of 26/104 (25%) patients experienced renal events based on both the 33% and 50% criteria (Table 11-9). Two patients required acute dialysis (defined as ≤ 40 days) for a mean of 1.5 days. Seven patients underwent chronic dialysis (defined as > 40 days), for a mean of 1055 days (median=597 days; SD=1067 days). One patient on chronic dialysis subsequently underwent a renal transplantation. A detailed analysis of renal event rate estimates based on serum creatinine and GFR is presented in Section 11.5.

Table 11-9: Mean Time (in Years) From Both Date of Birth and Qualification Start Date To Renal Event Occurrence in AGAL-008-00 Qualified Patients

	# Years from Qualification Start to Event (33% Criteria)	Age at Event (33% Criteria)	# Years from Qualification Start to Event (50% Criteria)	Age at Event (50% Criteria)
# Patients	26	26	26	26
Mean ± SD (yrs)	3.6 ± 3.58	43.6 ± 10.95	4.4 ± 3.68	44.3 ± 10.63
Median (yrs)	2.2	45.1	3.0	45.4
Min., Max. (yrs)	0.1, 13.8	18.1, 62.9	0.1, 13.8	19.9, 63.4
Reference: Table 14.7-2; Table 14.7-3				
Note: Event corresponds to increase in serum creatinine, dialysis, or transplantation.				

11.5 Estimation of Renal Event Rates Based on Serum Creatinine and GFR Modeling in the AGAL-008-00 Qualified Population

The overall objective of the analysis presented here is to apply statistical modeling to accurately estimate the rate of progression of renal disease in the AGAL-008-00 qualified population. The estimated rate in this historical population would then serve as a comparator for the active treatment group in the ongoing AGAL-008-00 clinical study and allow the conversion of AGAL-008-00 from a placebo controlled trial to a historically controlled, open label trial. This would allow all patients enrolled in the AGAL-008-00 trial to be converted to active treatment, thereby eliminating the concerns regarding feasibility of patient retention in a post-marketing setting and obviating the ethical concerns regarding a placebo-controlled post-marketing study in which the endpoint will likely result in irreversible end organ damage.

11.5.1 Renal Event Modeling

Summary tables and listings of renal, cardiac, cerebrovascular, and death data are presented in Appendix 16.2.9 for review.

11.5.2 Review of Renal Data

The historical control event rate was estimated by fitting statistical models to the set of patients who were in the AGAL-008-00 Qualified population. Certain entry criteria were made more stringent in these analyses in order to generate more meaningful estimates. Patients were required to have at least one serum creatinine value for the statistical modeling. In addition, the data after kidney transplantation and/or chronic dialysis (> 40 days), confounding renal disease, or enzyme replacement therapy were excluded. Two patients had extreme serum creatinine values of 12.0 or greater. For these two patients, observations collected after the first extreme measurement were outliers and excluded from the analysis.

The simplest method considered for estimating the renal event rate was the empirical estimate, based on tabulating the proportion of patients who were observed to have had a 33% increase in serum creatinine or otherwise who required intervention therapy (e.g., chronic dialysis and/or kidney transplantation) within the first 2 years of follow-up. However, this method failed to utilize all data. The data collected beyond the first 2 years of follow-up was not included in the empirical estimation of the event rate. Patients who had been followed for fewer than 2 years had a lower probability of event, and inclusion of these patients in the empirical estimation of event rate was biased (under-estimated). More precise and robust estimates of the event rate are obtained by modeling the underlying patient-specific trend in serum creatinine over the full duration of follow-up. Therefore, statistical models were used to estimate the event rate, and the logarithm of serum creatinine was modeled as a linear random effects model (Laird and Ware, 1982, Biometrics).

The model demonstrated that log-serum creatinine depended on the value at entry (intercept) and time since entry. The linear rate of change in log-serum creatinine over time (slope) depended on the patient. The model was fit by the method of restricted maximum likelihood, and the underlying patient-specific trend was determined from the empirical Bayes estimates of the random intercept and slope. On the log scale, the individual slope is directly related to the expected percent change in serum creatinine. The estimated historical control event rate was based on the random effects model fit to the Qualified population (n = 104). A slope of 0.143 translated to an expected 33% increase in serum creatinine within 2 years. The two-year event rate was estimated as the proportion of patients who had an estimated slope ≥ 0.143 . A slope of 0.135 translated to an expected 50% increase in serum creatinine within 3 years. The three-year event rate was estimated as the proportion of patients who had an estimated slope ≥ 0.135 .

The confidence interval for the historical control renal event rate point estimate is based on the confidence interval for the population mean slope, obtained from the fit of the random effects model. The confidence interval for the population mean slope is based on linear combinations of the restricted maximum likelihood estimates of the covariance parameters, and referenced to the approximate T-distribution. For the three-year event rate confidence interval the lower limit is the proportion of patients who had an estimated slope $\geq 0.135 + W$ where "W" is the half-width of the confidence interval for the overall population mean slope. The upper limit is the proportion of patients who had an estimated slope $\geq 0.135 - W$. The confidence interval for the two-year event rate is derived in a similar way.

To assess the robustness of the renal event rate estimates based upon the analysis of serum creatinine, the following supplementary analyses were performed:

- Dependence of the renal event rate on the entry criteria was investigated by fitting the random effects model to the larger patient populations defined by less rigorous entry criteria.
- Renal event rates were estimated based on the random effects model being fit to qualifying patients who had ≥ 2 observations and to qualifying patients who had ≥ 3 observations.
- The distribution of the estimated patient slopes and intercepts from the random effects model were reviewed.
- The distribution of the estimated patient slopes was reviewed by means of stratifying the estimated slopes based on subject covariates.
- Renal event rates were estimated based upon applying empirical methods.
- Renal event rates were estimated based on the random effects model with covariate terms included in the model.
- Renal event rates were estimated based on analysis of the data by means of individual patient regressions.
- A likelihood based approach to the renal event estimate.
- Exclusionary subset analyses.
- Analysis of 1/serum creatinine.

A detailed description of the linear random effects model and the method for estimating the event rate is presented in the statistical analysis plan (Appendix 16.1.9).

11.5.3 Historical Serum Creatinine Data

Serum creatinine levels are an important indicator of renal insufficiency in the Fabry population given that progressive GL-3 accumulation leads to loss of nephrons. Therefore, all available historical serum creatinine levels were collected. Table 11-10 summarizes the availability of serum creatinine records for AGAL-008-00 Qualified patients and the Total population.

Table 11-10: Serum Creatinine Records

Serum Creatinine Records Available	AGAL 008-00 Qualified Patients n (%)	Creatinine 1.2 – 3.0 (mg/dL) n (%)	All Patients n (%)
Total Patients	104/447 (23)	129/447 (29)	395*/447 (88)
1 record	19 (18)	24 (19)	107 (27)
2 records	21 (20)	21 (16)	83 (21)
3 – 5 records	26 (25)	27 (21)	96 (24)
> 5 records	38 (37)	57 (44)	109 (28)
* Patients with evaluable serum creatinine values. (Refer to Section 11.5.2) Reference: Table 14.8-2			

Certain additional populations were identified for analysis and event rate estimation based on the availability of their serum creatinine measurements (Table 11-11). Among AGAL-008-00 Qualified patients (N=104), 85/104 (82%) patients had ≥ 2 serial serum creatinine measurements, and 64/104 (62%) patients had ≥ 3 serial serum creatinine measurement. Based on these data, statistical models were generated that permitted event rate prediction for patients with any number of serum creatinine measurements.

Table 11-11: Populations Analyzed in Event Rate Estimation

Patient Groups	n (%)
Qualified Patients (i.e., met Inclusion/Exclusion Criteria of AGAL-008-00)	104 (100)
with ≥ 2 Serial Serum Creatinine Measurements	85 (82)
with ≥ 3 Serial Serum Creatinine Measurements	64 (62)
Reference: Table 14.8-2	

The reciprocal of serum creatinine is shown in Figure 11-2 for all AGAL-008-00 Qualified patients (linearized by taking the reciprocal of serum creatinine, i.e., 1/1.2 mg/dL = 0.83). For clarity, the patients are grouped according to slope/trend as determined by the random effects model (see Section 11.5.4). Figure 11-2 indicates that the majority of AGAL-008-00 Qualified patients show maintenance of current renal function (N=40) or worsening of renal insufficiency (N=33) based on their slope/trend. The results observed in the populations identified in Figure 11-2 become more robust by applying the requirement of three or more historical serum creatinine values Figure 11-3 (N=64). Again, the majority of patients show either maintenance of renal function (N=24) or a worsening of renal insufficiency (N=20). All graphs of serum creatinine comparisons are presented in Section 14.4.

Figure 11-2: Reciprocal of Serum Creatinine Data (1/serum creatinine) From All AGAL-008-00 Qualified Patients

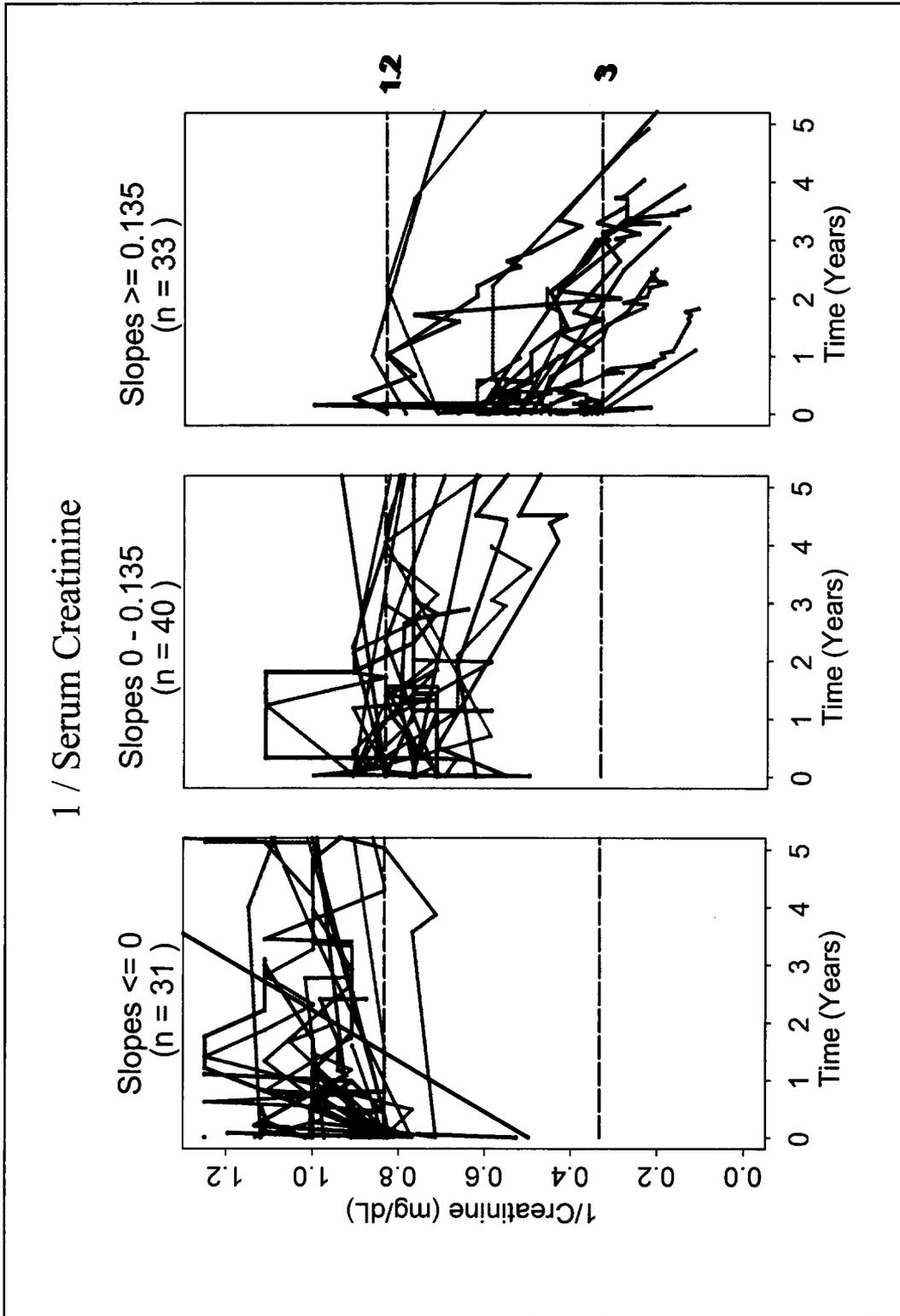
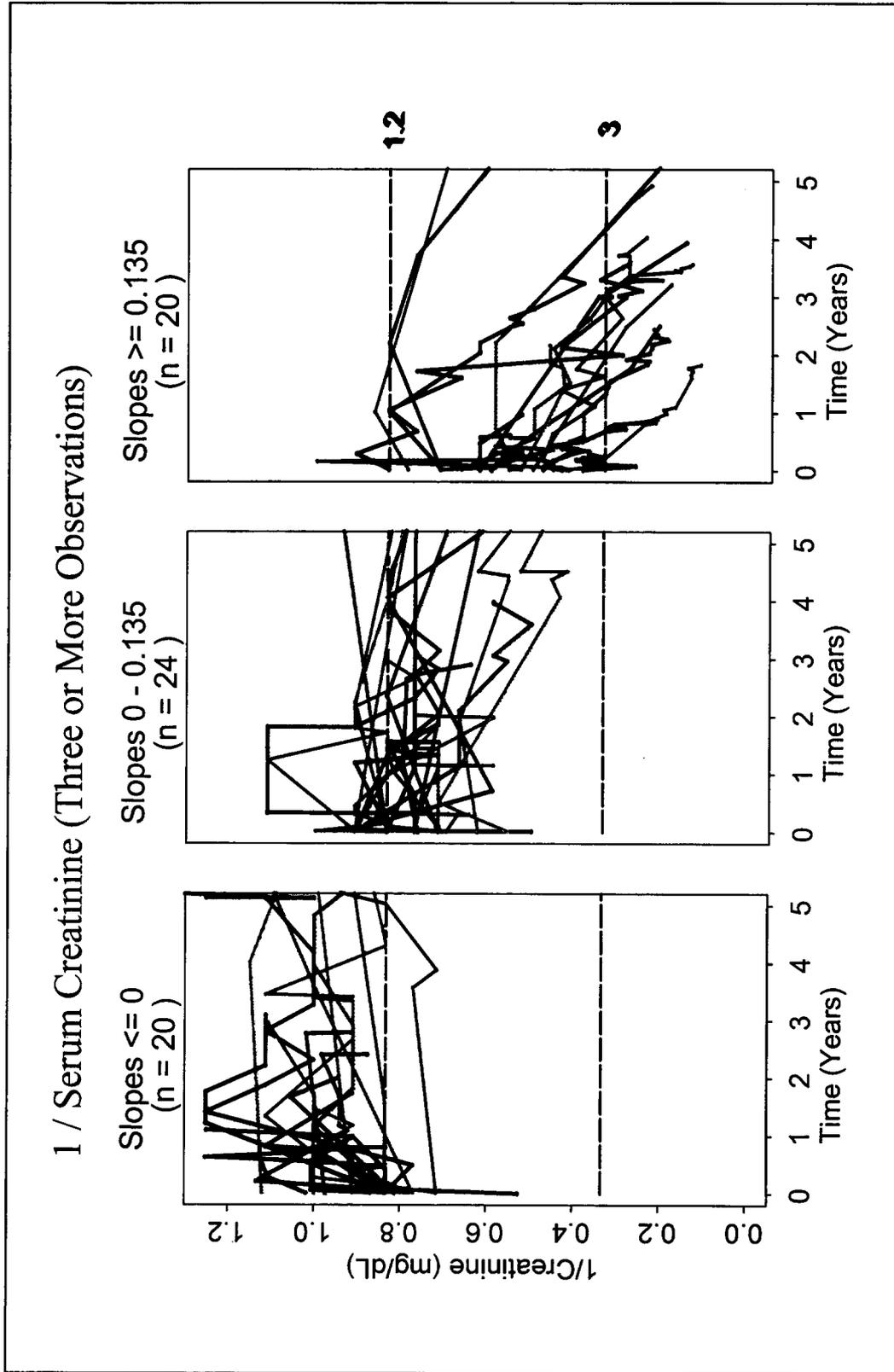
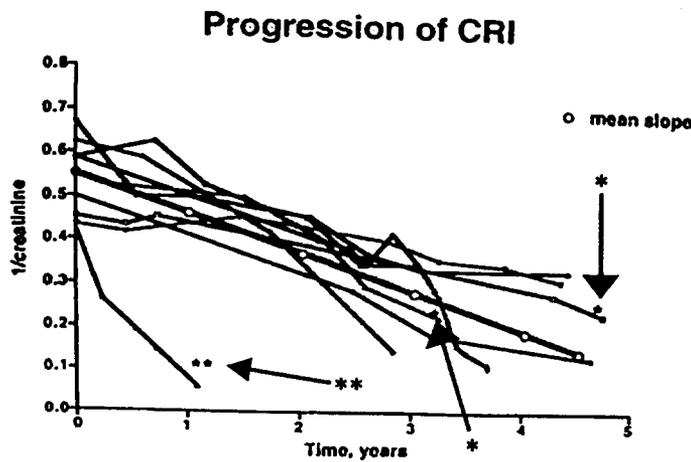


Figure 11-3: Reciprocal of Serum Creatinine Data (1/serum creatinine) From All AGAL-008-00 Qualified Patients with Three or More Serum Creatinine Observations



Collection of historical serum creatinine yielded robust data in line with a study conducted by Branton, (Branton, 2002, Medicine) that showed that after reaching a serum creatinine level of 1.5 mg/dL, Fabry patients' renal insufficiency rapidly worsened. From that study (Figure 11-4), 1/creatinine plots showed progression from earliest detected renal insufficiency to end-stage renal disease (ESRD). Three patients had hypertension prior to ESRD, 2 marginal or well-controlled (*), 1 poorly controlled (**). Despite the use of angiotensin-converting enzyme (ACE) inhibitors in three patients, all patients demonstrated progression of renal disease (i.e., a negative slope of 1/serum creatinine). Mean time from onset of chronic renal insufficiency to ESRD was 4.8 years. Mean rate of loss of GFR was 14 mL/min/year. Mean slope shown excludes outlier patient (**).

Figure 11-4: Longitudinal Data on Progression of Renal Insufficiency in Nine Patients with Fabry Disease (Branton, 2002, Medicine)



(Branton, 2002, Medicine)

11.5.4 Renal Event Rate Modeling and Estimation

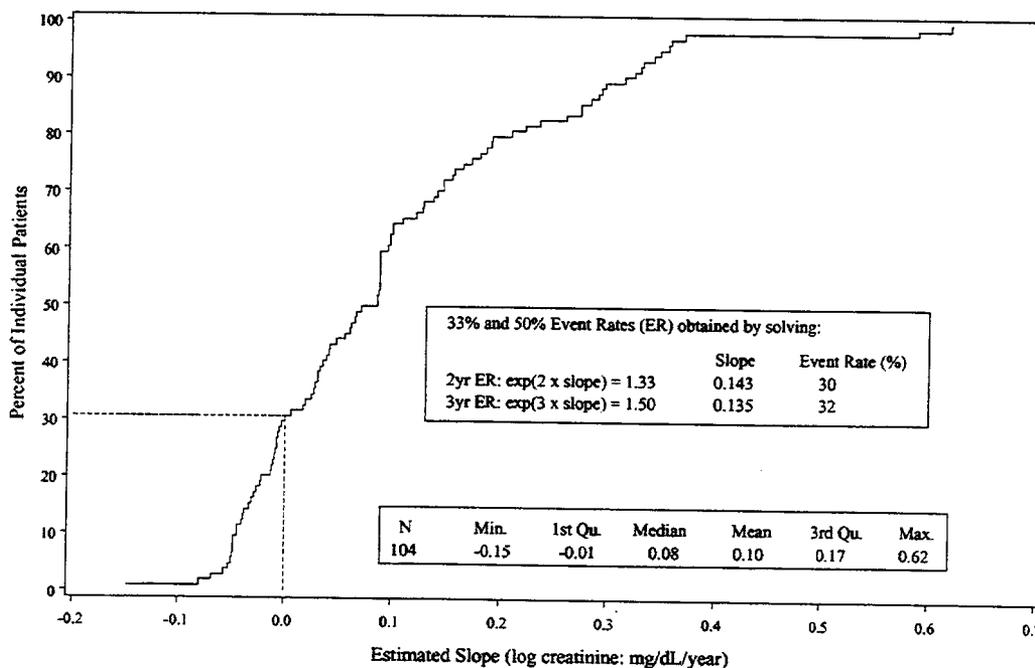
11.5.4.1 Event Rate Modeling and Estimation for the Qualified Population

Three hundred and ninety-five patients had at least one recorded measurement of serum creatinine in the database. One hundred and twenty-nine of these patients had at least one serum creatinine measurement in the range 1.2 – 3.0 mg/dL. One hundred and four patients fulfilled the entry criteria for the Phase 4 study (AGAL-008-00). The historical control renal event rate is based on fitting statistical models to the data of these 104 qualifying patients. The time of entry to the study is taken as the date upon which all entry criteria were first satisfied.

A linear random effects model was fit to the data of the 104 qualifying patients, using the method of restricted maximum likelihood estimation. Individual patient slopes were estimated using empirical Bayes estimation (Laird and Ware, 1982). The cumulative distribution of the estimated random slopes is displayed in Figure 11-5.

The mean and median one-year changes in log serum creatinine are 0.10 and 0.08 respectively. The patients who are expected to have a 33% increase in serum creatinine level within 2 years are the patients whose estimated slope is 0.143 or greater. Thirty-one patients have an estimated slope of 0.143 or greater. Seven of these patients required intervention therapy (i.e. chronic dialysis and/or transplantation) and their estimated slope exceeded 0.143. The estimated historical control event rate is 31/104 (30%) over two years. The patients who are expected to have a 50% increase in serum creatinine level within 3 years are the patients whose estimated slope is 0.135 or greater. Thirty-three patients have an estimated slope of 0.135 or greater. Seven of these patients required intervention therapy and their estimated slope exceeded 0.135. The estimated historical control event rate is 33/104 (32%) over three years (Figure 11-5).

Figure 11-5: Distribution for the Slope of Log Creatinine for Qualified Patients (n = 104)



11.5.4.2 Two- and Three-Year Event Rate Modeling and Estimation for the Qualified Population Stratified by Serum Creatinine Observations

The amount of data collected on a patient could possibly be related to disease progression. In order to examine the sensitivity of the estimated historical control event rate, linear random effects models were fit to the subsets of patients having two or more measurements of serum creatinine (n = 85) and the subset of patients having three or more measurements of serum creatinine (n = 64). (Figure 11-6 and Figure 11-7) The two- and three-year event rates derived from these models fall in the range 28 – 31% and the results are summarized in Table 11-12. The

estimated historical control two- and three-year event rates are not sensitive to the amount of data collected on each patient.

Table 11-12: Estimated Event Rate for AGAL-008-00 Qualified Population

		Two Year Event Rate		Three Year Event Rate	
	Number of Patients	Number With Slope ≥ 0.143	Event Rate %	Number With Slope ≥ 0.135	Event Rate %
All Qualified	104	31	30 (31/104)	33	32 (33/104)
≥ 2 observations	85	24	28 (24/85)	25	29 (25/85)
≥ 3 observations	64	19	30 (19/64)	20	31 (20/64)
Reference: Table 14.8-3					

The two- and three-year event rates for both subsets of patients were similar to the two- and three-year event rates calculated for the Qualified population (n = 104). These results demonstrate that the model is robust. (Figure 11-6 and Figure 11-7)

Figure 11-6: Distribution for the Slope of Log Creatinine for AGAL 008-00 Qualified Patients with Two or More Serum Creatinine Observations (n = 85)

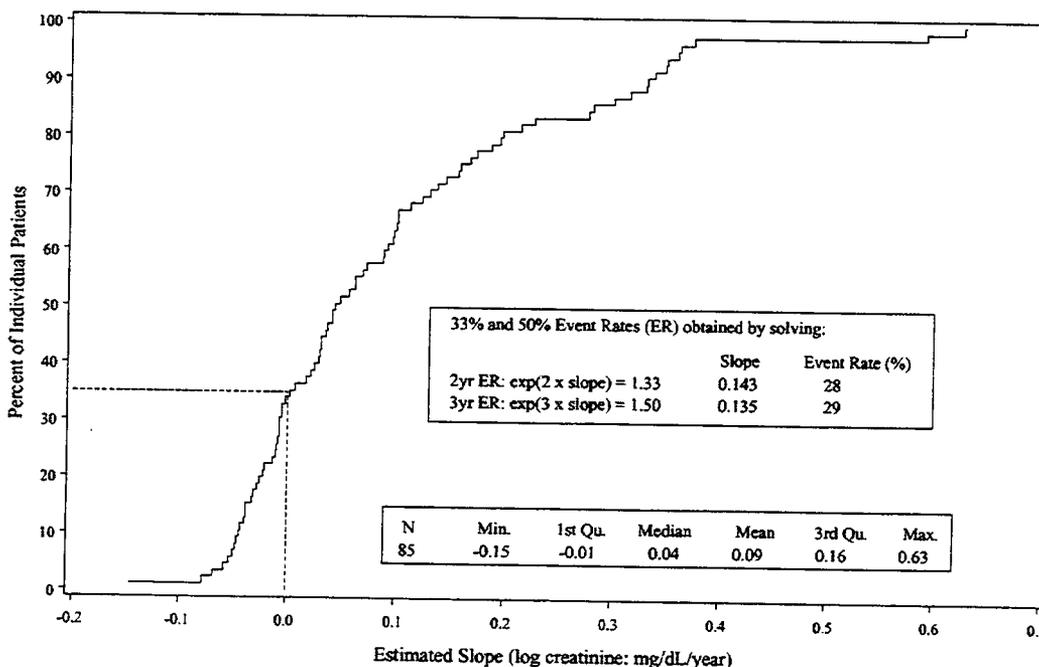
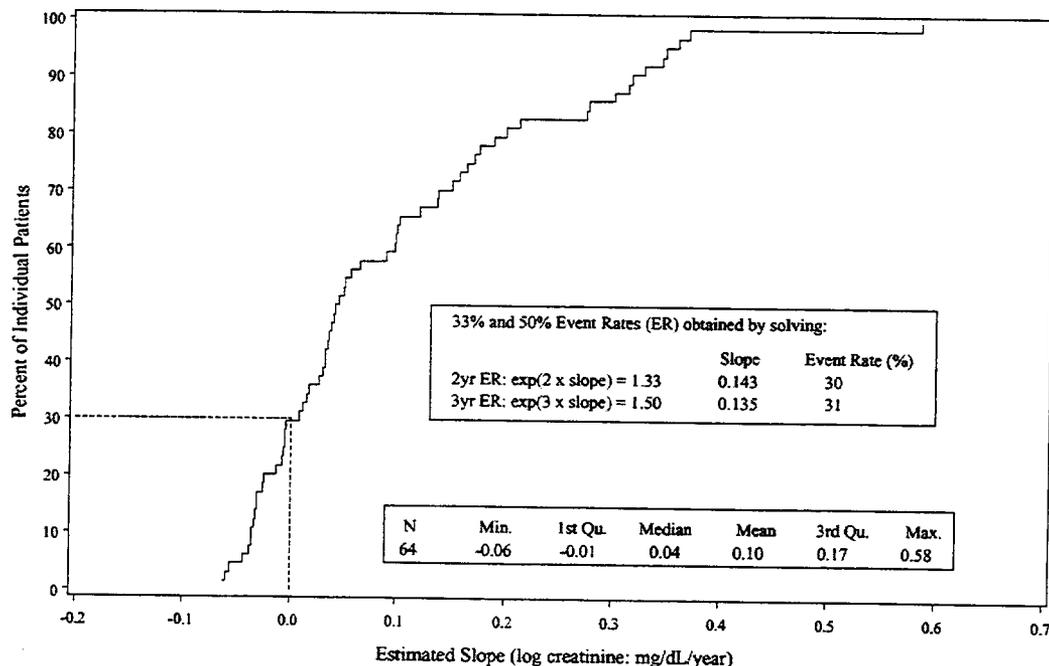
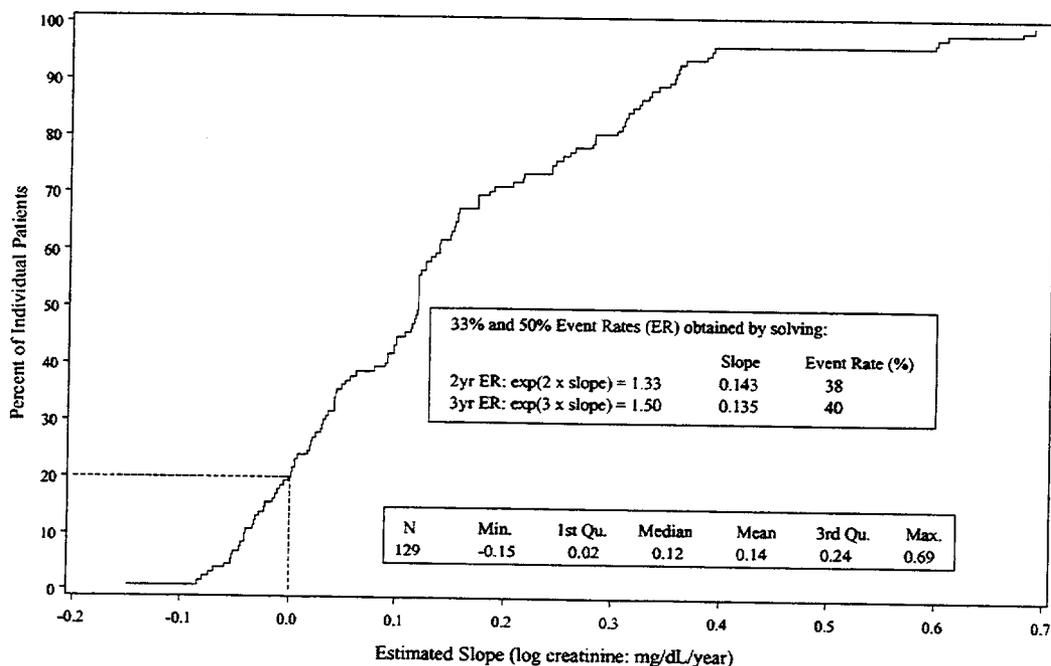


Figure 11-7: Distribution for the Slope of Log Creatinine for AGAL-008-00 Qualified Patients with Three or More Serum Creatinine Observations (n = 64)



The model was fit to a population with serum creatinine levels ≥ 1.2 mg/dL and < 3.0 mg/dL and the corresponding estimate of event rate was somewhat higher. (Figure 11-8)

Figure 11-8: Distribution for the Slope of Log Creatinine for Patients with At Least One Serum Creatinine Measurement Between 1.2 – 3.0 mg/dL (n = 129)



The estimated two-year event rate for the 129 patients who had at least one serum creatinine measurement in the range 1.2 – 3.0 was 38%.

All graphs of event rate modeling are presented in Section 14.4.

Table 11-13 summarizes the two- and three-year event rates displayed in the figures according to serum creatinine level increases of 33% and 50%.

Table 11-13: Estimated Event Rate for Renal Events Based on AGAL-008-00 Qualified Population (n = 104) and the Random Effects Model

Serum Creatinine Increase	Estimated Event Rate
33% Increase Over 2 Years	30%
50% Increase Over 3 Years	32%
Reference: Table 14.8-3	

It should be noted that compared to the first interim report, the estimated 2 year and 3 year renal event rates have declined. From the time of the first interim report there has been an increase of 61 patients included in the AGAL-008-00 Qualified population (n=43 vs. n=104 respectively). The median age of the AGAL-008-00 Qualified patients has decreased from the time of the first interim report from a median age of 39.8 to 37.2 in this report and median serum creatinine values have changed from 1.4 to 1.3 mg/dL.

11.5.4.3 Empirical Estimation of Renal Event Rates

To explore whether the estimated renal event rates depend on the random-effects model assumptions, a comparison was run against an alternate estimation of event rate which is free of the assumptions of the random effects model: the empirical estimation method.

The empirical 2-Year event rate is based on determining the number of patients who have a serum creatinine measurement observed 2 years after the initial (qualifying) measurement, and computing the proportion of such patients whose 2-Year measurement is at least 33% greater than the measurement made at the initial visit. The empirical 3-Year event rate is determined in the same way, based on a 50% increase in serum creatinine levels at 3 years after the initial (qualifying) measurement.

For the historical database there is irregularity in the timing of serum creatinine measurements and variation in the duration of follow-up among patients. Few patients have measurements at precisely 2 years following the initial measurement and the empirical estimate of renal event rate is unable to make use of all data. In order that the empirical estimates are based on sufficiently large denominators, a one-year window was used. The empirical 2-Year event rate is based on determining the group of patients who have a serum creatinine measurement observed between 2

and 3 years following the initial measurement. The empirical 2-Year event rate based on an interval centered at 2 years: 1.5 to 2.5 year window was also evaluated.

Table 11-14 provides the empirical estimates of renal event rate for patients having at least one measurement available in the time intervals: 1 - 2 years, 2 - 3 years, and 3 - 4 years. Table 11-15 provides the empirical estimates of renal event rate for patients having at least one measurement available in the time intervals: 0.5 - 1.5 years, 1.5 - 2.5 years, and 2.5 - 3.5 years.

The estimated 2-Year empirical event rates (Table 11-14 and Table 11-15) are 31% and 25% and are similar to the estimated 2-Year rate based on the random-effects model (30%, see Table 11-13). The estimated 3-Year empirical event rates (Table 11-14 and Table 11-15) are 41%. These estimated 3-Year rates are slightly larger than the estimated 3-Year rate based on the random-effects model (32%, Table 11-13), however, the empirical estimates at 3-Years are highly variable since they are based on data from only a limited number of patients (17 patients).

Table 11-14: Estimates of Renal Event Rate Based on the Empirical Method

	1 - 2 Years	2 - 3 Years	3 - 4 Years
Number of Patients	35	26	17
33% Serum Creatinine Increase	8 (23%)	8 (31%)	7 (41%)
50% Serum Creatinine Increase	7 (20%)	6 (23%)	7 (41%)
Reference: Table 14.8-8			

Table 11-15: Estimates of Renal Event Rate Based on the Empirical Method (Centered Intervals)

	0.5 - 1.5 Years	1.5 - 2.5 Years	2.5 - 3.5 Years
Number of Patients	44	32	17
33% Serum Creatinine Increase	8 (18%)	8 (25%)	7 (41%)
50% Serum Creatinine Increase	5 (11%)	6 (19%)	7 (41%)
Reference: Table 14.8-8			

The random-effects model allows for estimation of renal event rates using all the available data; therefore this method provides a better estimate of the true event rate. It should be noted that the empirical estimates of renal event rate are comparable to the estimates of renal event rate based on the random-effects model, thereby supporting the use of the random-effects model.

11.5.4.4 Justification of the Logarithmic Transformation

Trends in the logarithm of serum creatinine are assumed linear in observation time. Two key assumptions of the random-effects model enable use of all data for estimating the renal event rate.

- Linear trend in the population mean log serum creatinine over time.
- Normal distribution for the random error.

Figure 11-9 displays a scatter-plot of serum creatinine measurement and the time of measurement post initial visit. The distribution of serum creatinine is highly skewed with many extreme-high values.

Figure 11-9: Distribution of Serum Creatinine for Qualified Patients over Time

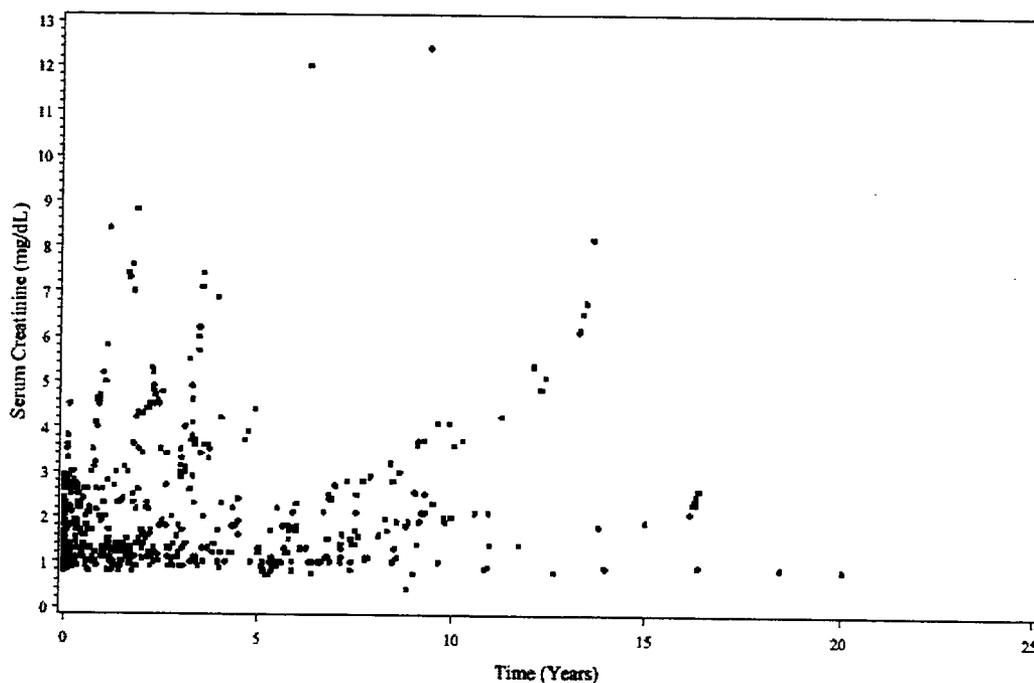


Figure 11-10 presents the scatter-plot based on the natural logarithmic transformation (\log) of serum creatinine. The data appear close to symmetric and there are no gross outlying observations. The trend in \log serum creatinine is not clear from the scatter-plot. Figure 11-11 presents the scatter-plot of \log serum creatinine for the first five years of observations. A lowess curve (robust locally weighted regression and smoothing scatterplots) (Cleveland, 1979) has been fit through the data. The lowess curve is a moving average estimate of the mean, where observations closest to the center of the moving window are weighted more heavily than observations lying further from the center of the moving window. The graph is restricted to the first 5 years of observation time, since few patients have data after 5 years and these patients could unduly influence the lowess estimate of means at early time points. Renal events are

defined based on 2 or 3 years of observation, and measurements past 5 years are less relevant. The lowess curve is reasonably linear, supporting a linear relationship between time of observation and log serum creatinine.

There appear to be no large deviations from the underlying assumptions of the random-effects model. Therefore, it is expected that the Empirical Bayes estimates of individual patient trends fit closely to the observed patient data, and the calculated estimate of renal event rates based on the Empirical Bayes fits should be valid. Individual plots of observed patient data and corresponding Empirical Bayes estimates are provided in Section 14.4. The Empirical Bayes estimates fit closely to the data of most patients.

Figure 11-10: Distribution of the Log Serum Creatinine for Qualified Patients over Time

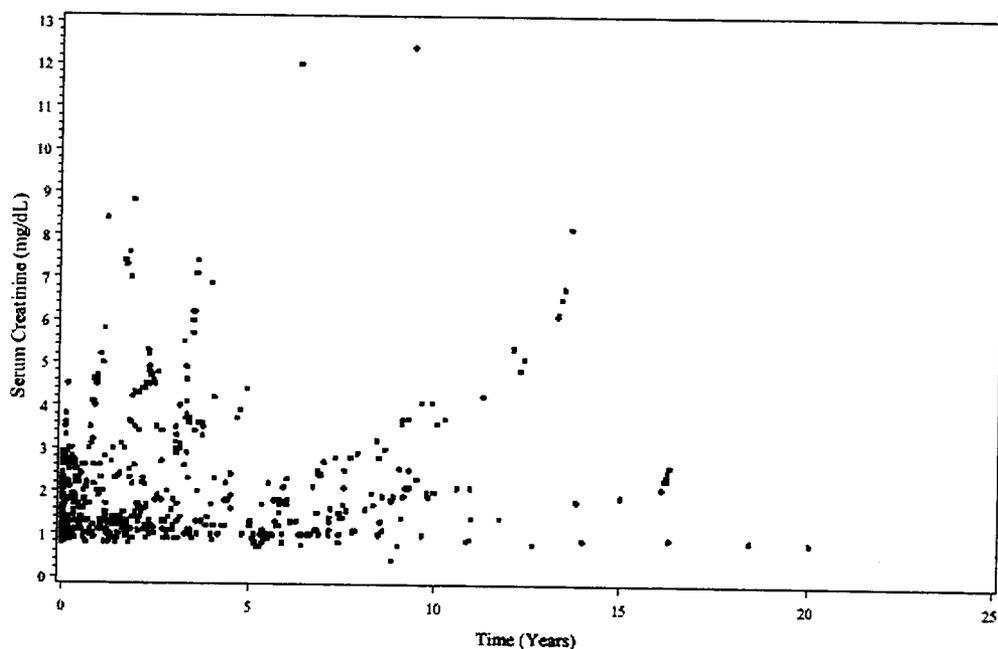
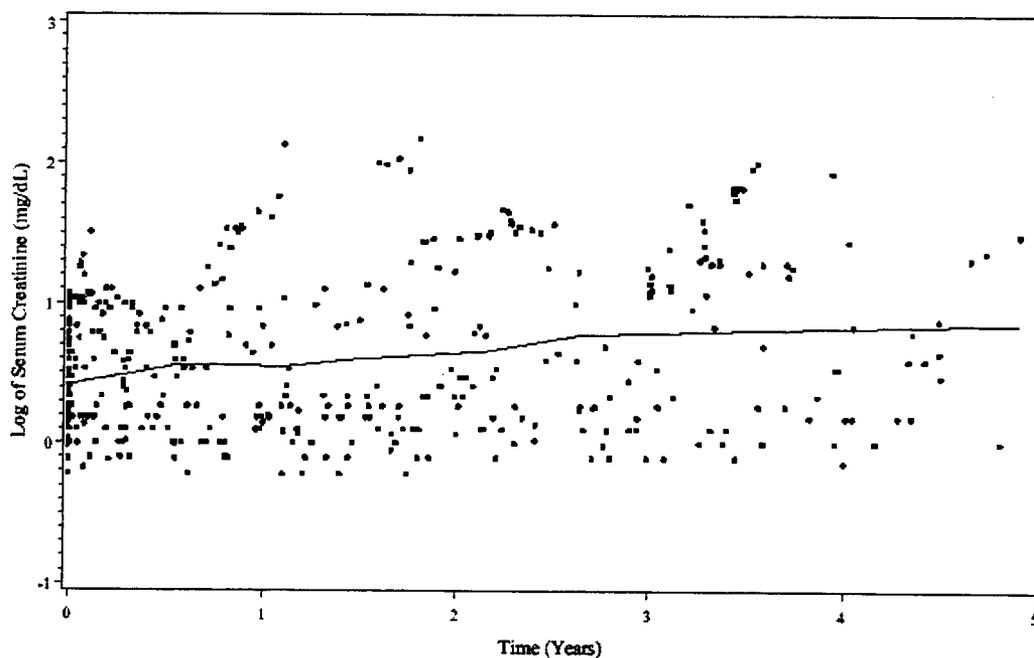


Figure 11-11: Distribution of the Log of Serum Creatinine for Qualified Patients over First 5 Years of Observation Time



A log scale was chosen for modeling serum creatinine since the Empirical Bayes estimates from the linear trend random-effects model fit closely to the observed patient data. A close fit is possible as the distribution of the log serum creatinine measurements do not depart far from the Normal distribution and the trend of mean log serum creatinine does not depart far from linear.

The scatter-plots of log serum creatinine and lowess curves (Figure 11-9, Figure 11-10, and Figure 11-11) suggest that the linear trend random effects model will fit closely to log serum creatinine. The individual data and Empirical Bayes fits (Section 14.4) confirm that the model fits well. The data of a few patients suggest possible quadratic trends. As a sensitivity analysis, alternative random effects models are fit to the data.

Table 11-16 presents the estimated fixed-effects for 3 random effects models fit to the log of serum creatinine:

- Linear $(a + a_i) + (bT + b_iT)$
- Quadratic $(a + a_i) + (bT + b_iT) + (cT^2 + c_iT^2)$
- Cubic $(a + a_i) + (bT + b_iT) + (cT^2 + c_iT^2) + dT^3$

Table 11-16: Linear, Quadratic, and Cubic Trend Random-Effects Model Fits

	Estimate	SE	DF	t-value	p-value
Linear Trend					
Intercept	0.3060	0.0310	103	9.88	<0.001
Time	0.0998	0.0219	84	4.55	<0.001
Quadratic Trend					
Intercept	0.3386	0.0292	103	11.58	<0.001
Time	0.0291	0.0215	84	1.36	0.179
Time ²	0.0189	0.0043	64	4.34	<0.0001
Cubic Trend					
Intercept	0.3400	0.0292	103	11.64	<0.001
Time	0.0231	0.0228	84	1.01	0.314
Time ²	0.0207	0.0049	64	4.27	<0.001
Time ³	-0.0001	0.0001	360	-0.94	0.346

Reference: Table 14.8-10

The cubic trend model does not include a cubic random-effect. The cubic fixed-effect term is not significant and a cubic trend model with cubic random-effect was not explored. The quadratic trend model has a significant quadratic fixed-effect. However, due to the high degree of correlation among the polynomial trend estimates, misinterpretation of individual trend estimates is possible.

Table 11-17 presents the estimated renal event rates based on the linear trend and quadratic trend random-effects models. The first row of the table gives the event rate based on fitting the model to all data. Subsequent rows are based on fitting the models to smaller observation time windows.

Table 11-17: Estimated Renal Event Rates Based on the Linear and Quadratic Trend Random-effects Models with Time-Subsets of Data

Time of Observation	Linear Trend		Quadratic Trend	
	2 Year Event Rate (%)	3 Year Event Rate (%)	2 Year Event Rate (%)	3 Year Event Rate (%)
All Data	29.8	31.7	25.0	28.8
≤ 15 Years	29.8	31.7	25.0	28.8
≤ 13 Years	29.8	30.8	25.0	28.8
≤ 11 Years	30.8	30.8	25.0	28.8
≤ 10 Years	30.8	30.8	25.0	28.8
≤ 9 Years	28.8	29.8	25.0	29.8
≤ 8 Years	30.8	30.8	26.9	30.8
≤ 7 Years	30.8	30.8	26.9	29.8
≤ 6 Years	30.8	30.8	26.9	30.8
≤ 5 Years	30.8	31.7	26.0	29.8
≤ 4 Years	29.8	30.8	26.0	31.7
≤ 3 Years	28.8	28.8	25.0	38.5
≤ 2 Years	26.9	29.8	32.7	61.5

Reference: Table 14.8-11

The estimated 3-Year renal event rates for the linear and quadratic trend models are similar. The 3-Year rates for the linear trend model range from 28.8% to 31.7% and the 3-Year rates for the quadratic trend model range from 28.8% to 61.5%. The 3-Year renal event rate for the quadratic trend model, based on observations collected within the first 2 years is 61.5%. This extremely high rate suggests that the quadratic trend model does not extrapolate well.

The estimated 2-Year event rates based on the quadratic trend model range from 25.0 to 32.7% and are somewhat lower than the 2-Year event rates based on the linear trend model (26.9-30.8%).

The quadratic term in the quadratic trend model is statistically significant, and so the quadratic trend model has a better fit to the data than the linear trend model. The two models yield similar estimated renal event rates. The linear trend model was favored since it is simpler, biologically feasible (exponential growth) and easier to explain. The quadratic trend model means become unreasonable at large observation times.

The estimate of the renal event rate is based on two steps. First a random-effects model is fit to the serum creatinine data in order to obtain Empirical Bayes estimates of trend in serum creatinine for each individual patient. The renal event rate is then calculated as the proportion of patients who have an estimated 33% (50%) increase in serum creatinine over 2 years (3 years). The random-effects model-based estimate of renal event rate uses all patient data and the validity of the estimate depends on the Empirical Bayes estimates fitting closely to the individual patient data.

The distribution for serum creatinine is skewed towards extreme high values and violates the Normal distribution assumption of the random-effects model. The distribution of log serum creatinine does not depart far from the Normal distribution and the relationship between observation time and log serum creatinine does not depart far from the linear relationship assumed by the random-effects model. The assumptions of the linear trend random-effects model for log serum creatinine are not violated and the Empirical Bayes estimates fit closely to the individual patient data.

The quadratic trend random-effects model for log serum creatinine also fits well to the data. The 3-Year renal event rate estimates based on the linear and quadratic trend random-effects models are similar. The 2-Year renal event rate estimate based on the quadratic trend random-effects models is slightly lower than the estimate based on the linear trend random-effects model.

The linear random effects model was chosen as the primary model for estimate of the renal event rate. This is based on the model fitting the data, it is relatively simple, biologically feasible (exponential growth), and easy to explain. The slope parameter of the linear trend model of log serum creatinine is directly related to percent change in serum creatinine.

11.5.4.5 Distribution of the Estimates Slopes and Intercepts

To assess if the normal distributional assumptions hold and whether there may be two distinct patient populations in the AGAL-014-01 Historical study population, scatter-plots of the patient random intercepts and slopes are presented in Figure 11-12, Figure 11-13, and Figure 11-14. The figures are based on the random effects model fitted to: all AGAL-008-00 Qualifier patients, AGAL-008-00 Qualifier patients who have ≥ 2 observations, AGAL-008-00 Qualifier patients who have ≥ 3 observations.

Figure 11-12: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients

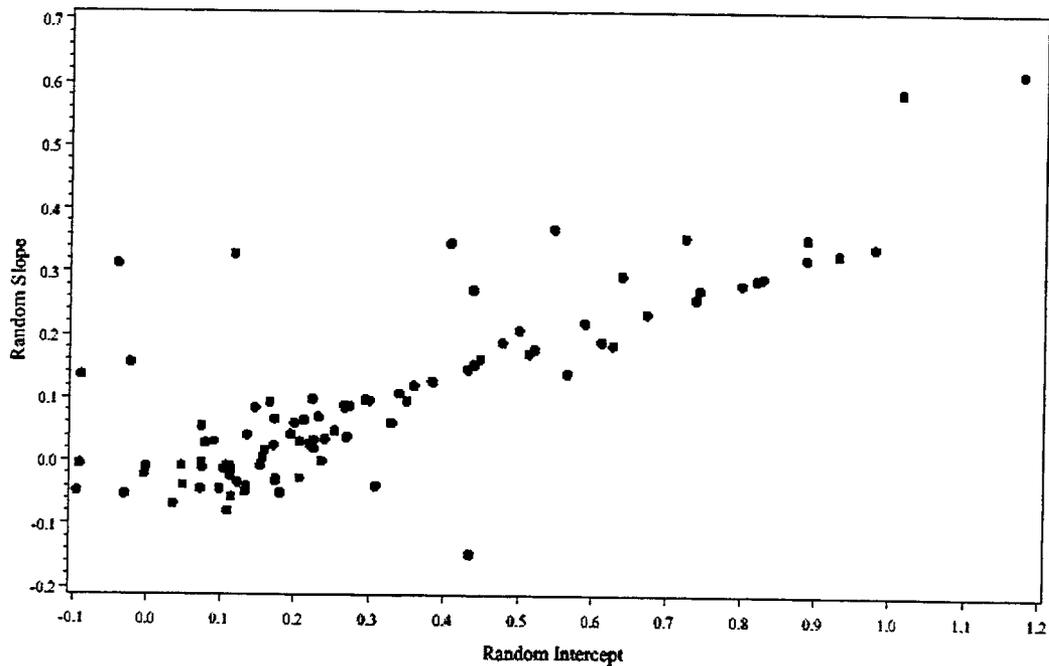


Figure 11-13: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients with Two or More Serum Creatinine Records

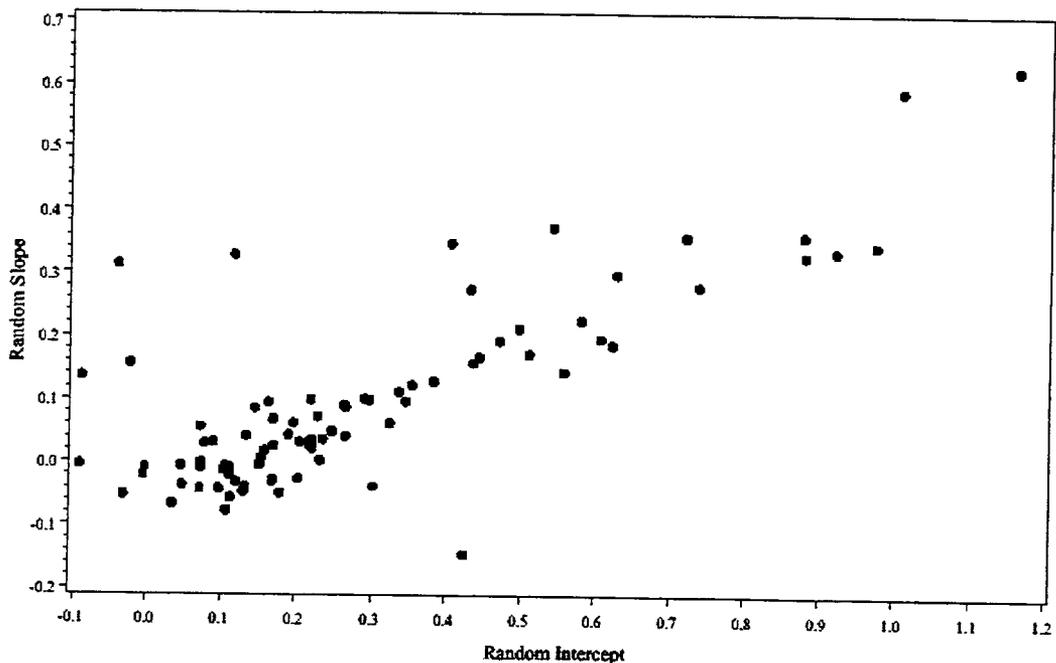
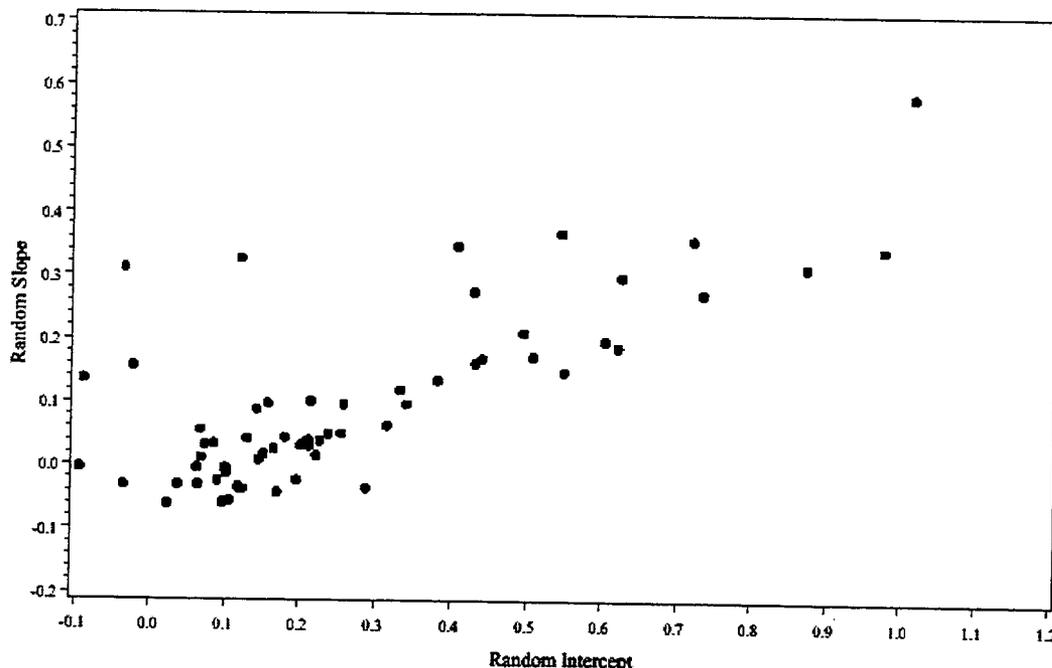


Figure 11-14: Scatter-Plot of the Estimated Subject Intercepts and Slopes Based on Serum Creatinine Random Effects Analysis for Qualified Patients with Three or More Serum Creatinine Records



The scatter-plots seem consistent with a sample from a bivariate normal distribution (single population). The cloud of points is roughly elliptical in shape. There is a positive correlation between intercept and slope, indicating that patients with high initial serum creatinine values tend to have a larger slope of log serum creatinine than patients with low initial serum creatinine values. There is skewness in the distribution of random intercepts and slopes with heavier mass toward low values for intercepts and slopes.

11.5.4.6 Adjustments for Covariates

To assess the robustness of the model and analysis results, selected covariates were individually added as fixed effects to the random effects model. Table 11-18 summarizes the results of these supplementary analyses. The covariate parameter estimate, p-value, and the estimate of the renal event rate are presented.

Table 11-18: Parameter Estimates for Selected Covariates and Adjusted Renal Event Rate

Covariate Included	Covariate Description	Parameter Estimate	p-value	Adjusted 33% Renal Event Rate*	Adjusted 50% Renal Event Rate*
Age	Age at Qualification Date	0.0028	0.336	27.88 (29/104)	30.77 (32/104)
Gender	Dichotomous: Male/Female	0.1598	0.215	29.81 (31/104)	31.73 (33/104)
Weight	Weight at Qualification Date	0.0069	0.008	32.18 (28/87)	32.18 (28/87)
Blood Type	Dichotomous: {A+, A-, O+, O-} or {B+, B-, AB+, AB-}	-0.1229	0.190	26.87 (18/67)	26.87 (18/67)
Plasma a-GAL	Plasma a-GAL level	-0.0548	0.426	26.79 (15/56)	28.57 (16/56)
ACE Inhibitor Use	- Prior to Qualification Start Date - On or After Qual. Start Date - No ACE Inhibitor Data Recorded	0.0918 -0.1027	0.289 0.158	27.88 (29/104)	30.77 (32/104)

Note: Renal event rate = (# estimated renal events/# patients) based on a 50% increase serum creatinine over 3- years (corresponding to a slope cut- off of 0.135) and based on a 33% increase serum creatinine over 2- years (corresponding to a slope cut- off of 0.143).

Note: ACE Inhibitor use incorporates ACE Inhibitors and ARBs (ACE Receptor Blockers).

Reference: Table 14.8-5

The results of the covariate analyses support the estimated renal event rate as determined by the random effects model. The inclusion of these covariates into the model did not appreciably affect the estimated renal event rate. To further assess the results of the random effects model, the distribution of the estimated subject slopes were examined by stratifying the values based on selected covariates. For continuous covariates, quartile stratification will be used (Q1: 25th percentile, Q2: 50th percentile, Q3: 75th percentile). The covariates are:

Age: stratified by quartiles

Gender: stratified by male/female

Weight: stratified by quartiles

Blood Type: stratified by {B-, B+, AB-, AB+}, {A-, A+, O-, O+}

Plasma αGAL: stratified by quartiles

Baseline (i.e., Qualification Start Date) Serum Creatinine: stratified by quartiles

ACE Inhibitor Use: Stratified by “Prior to Qualification Start Date” (Patients started on ACE inhibitors prior to Qualification Start Date), “On or After Qualification Start Date”, and “No ACE Inhibitor Data Recorded”

For each covariate stratification analysis, the number of subject slopes, median, mean, and standard deviation are presented (See Section 14.4). In addition, a box and whiskers plot is produced for each of the covariate groups (Figure 11-15, Figure 11-16, Figure 11-17, Figure 11-18, Figure 11-19, Figure 11-20, and Figure 11-21).

Figure 11-15: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Plasma α-GAL

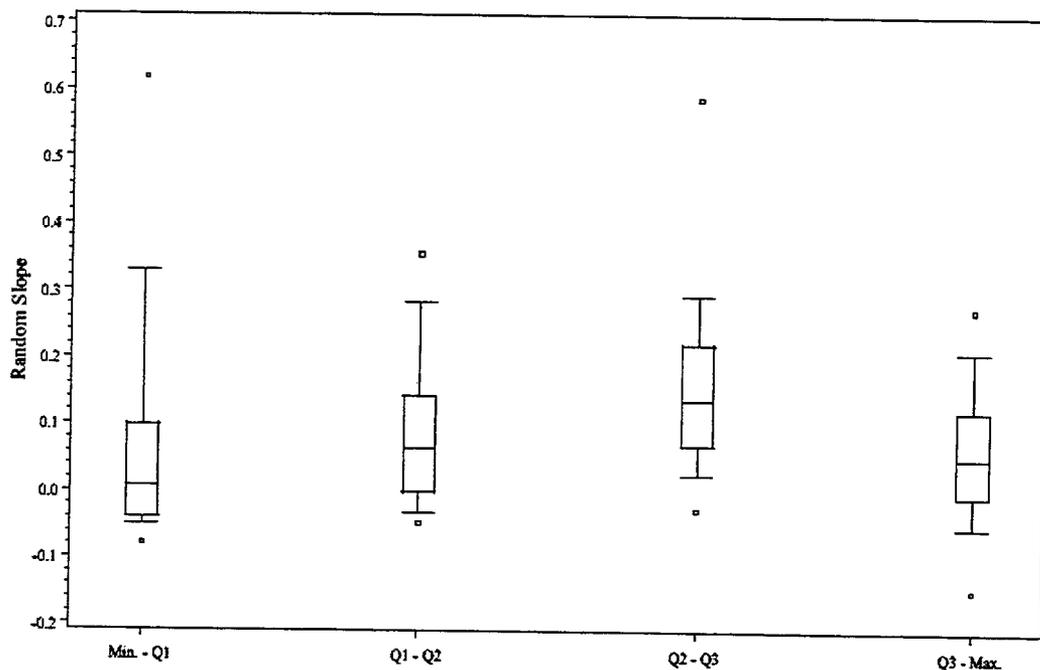


Figure 11-16: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Age at Qualification Quartiles

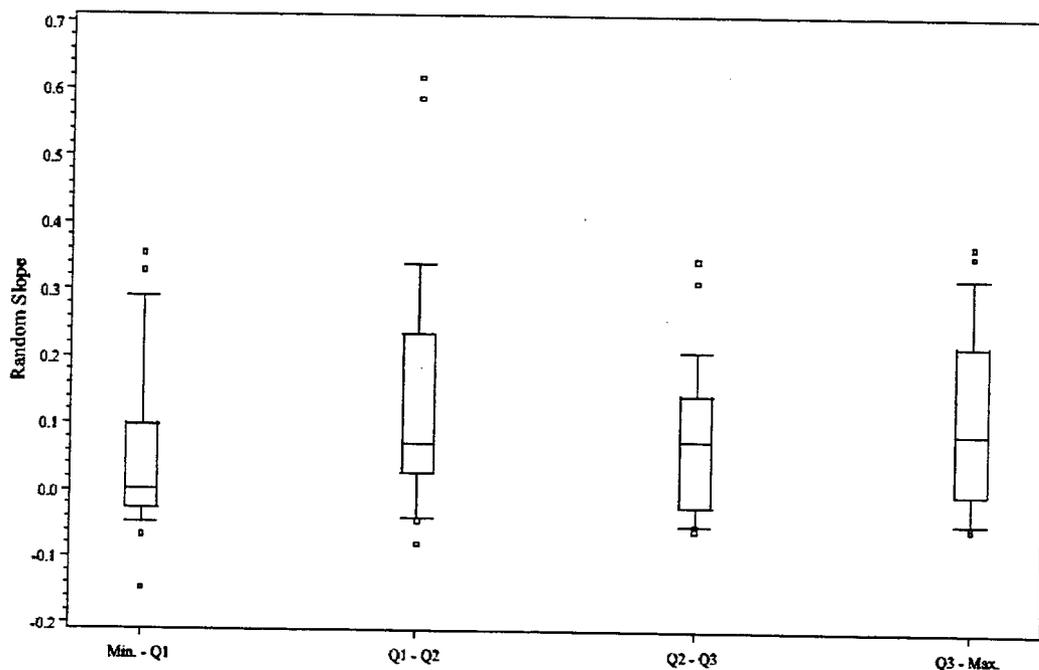


Figure 11-17: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Serum Creatinine at Qualification Quartiles

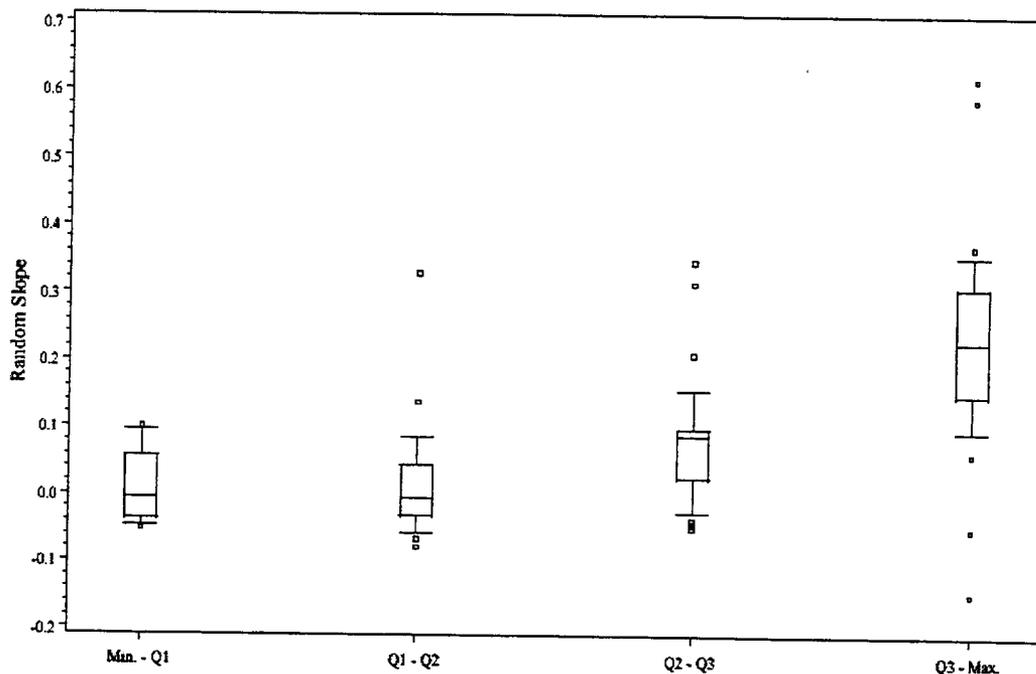


Figure 11-18: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Weight at Qualification Quartiles

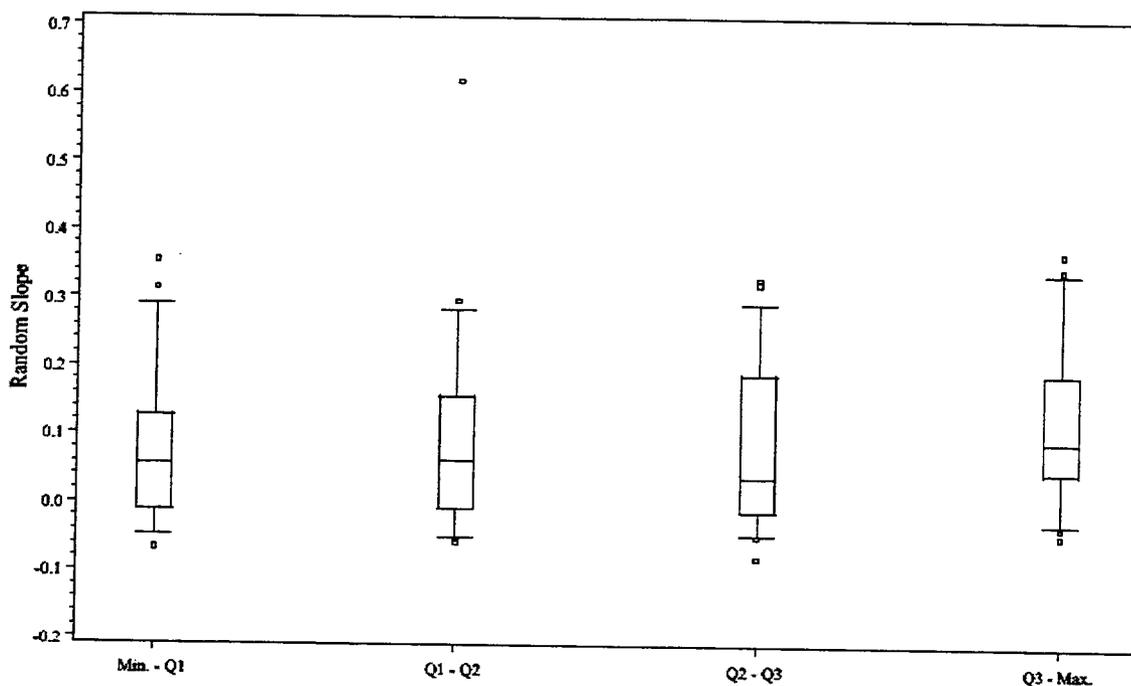


Figure 11-19: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Blood Type

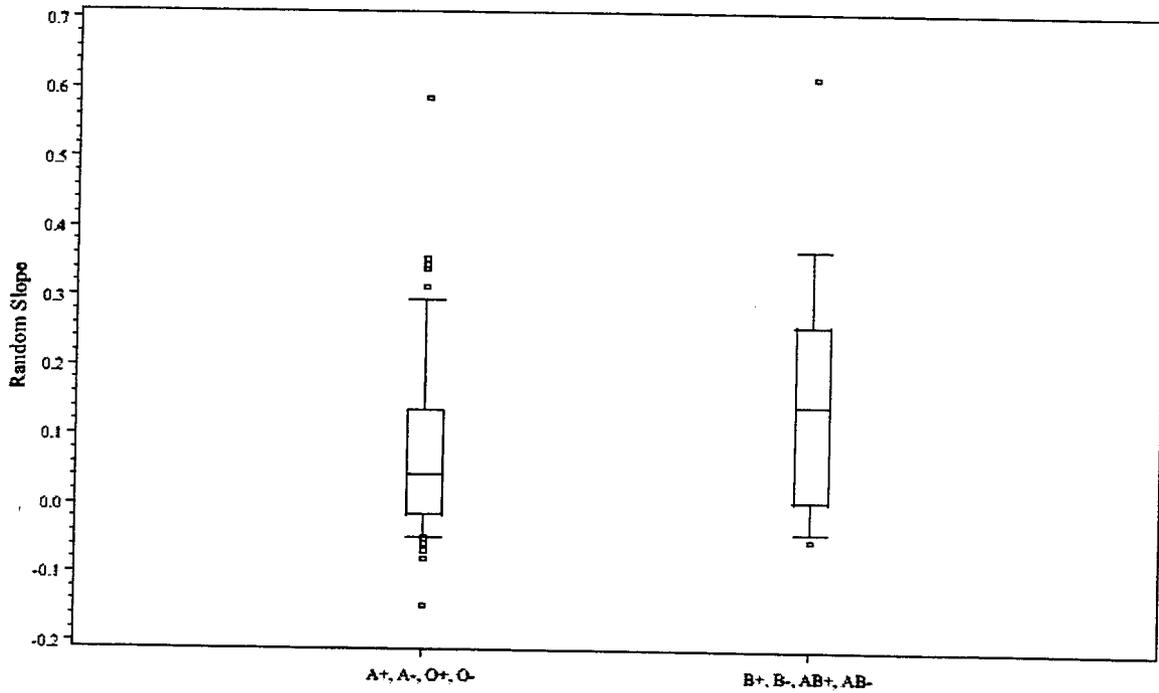


Figure 11-20: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by Gender

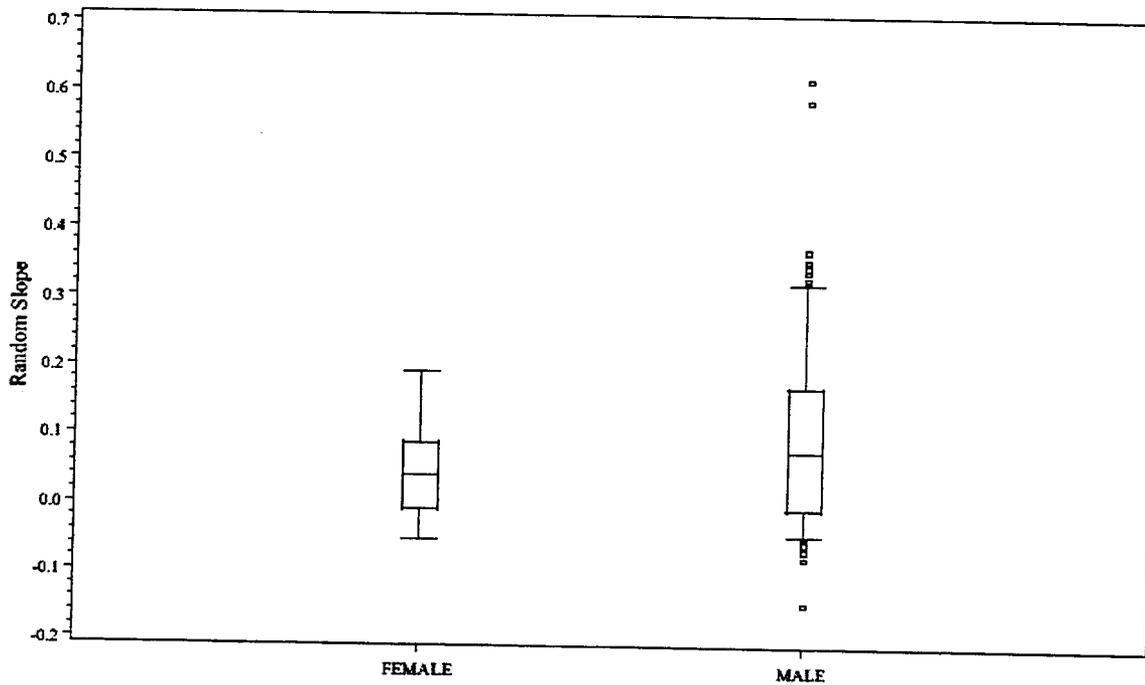
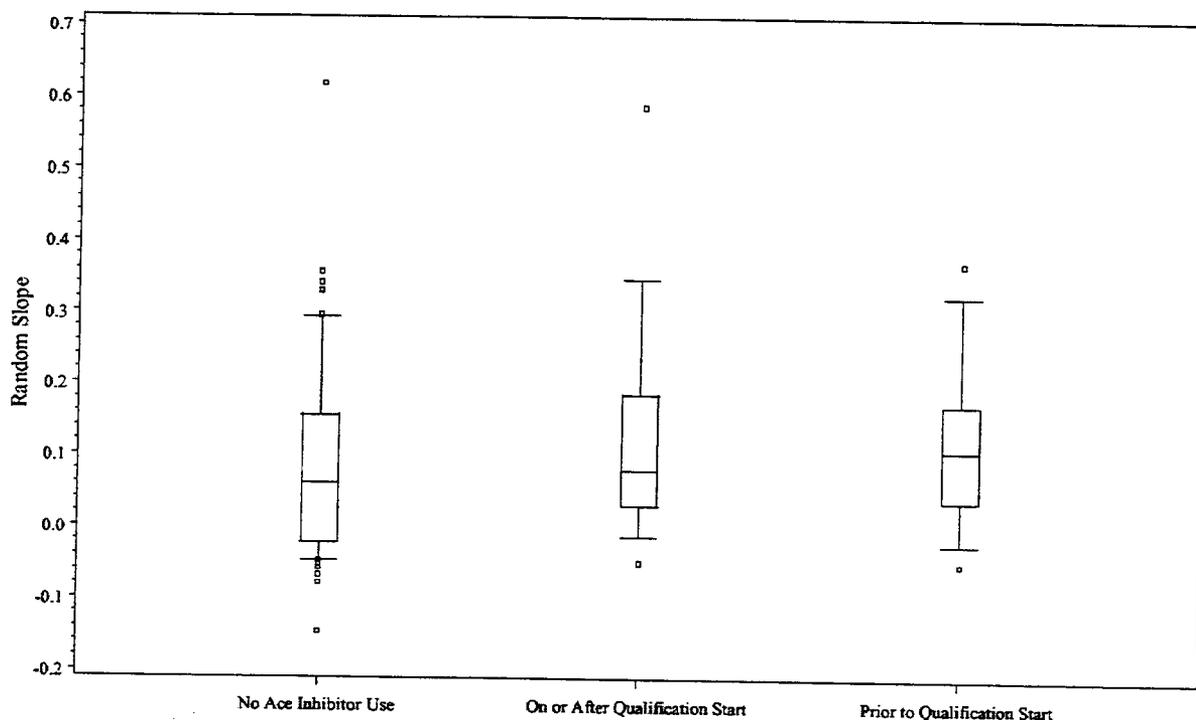


Figure 11-21: Distribution of Patient Slopes from Random Effects Model of Serum Creatinine Stratified by ACE Inhibitor Use



The results of the stratification of the subject slopes by selected covariates do not indicate any fundamental inadequacies in the random effects model. The covariate stratification of: age, gender, weight, and plasma α GAL do not indicate a trend in the subject slopes. There is a small difference in subject slopes relative to blood types, but it was seen in the covariate analysis that this difference was not statistically significant and did not affect the results of the estimated renal event rate. A positive correlation between baseline (i.e., Qualification Start Date) serum creatinine value and subject slopes is exhibited in the data. This positive correlation between the baseline serum creatinine value and the slope is incorporated into the parameterization of the random effects model.

The results of these analyses support that the random effects model is appropriate for the estimation of renal event rates and demonstrate the robustness of the results.

11.5.4.7 By Subject Linear Regression

To further assess the results from the random effects model, three individual regression models were fit to each subject's log serum creatinine data. These models were:

- *An intercept only regression model (based on patients with $n \geq 1$ observations)*
- *A linear regression model (with a linear and intercept term, based on patients with $n \geq 2$ observations)*
- *A quadratic regression model (with a quadratic, linear, and intercept term, based on patients with $n \geq 3$ observations).*

For each subject, the sum of squared residuals (SSR) from each of the three regression models and the random effects model are provided in electronic data format. With respect to the sum of squared residuals adding terms to a regression model can only decrease the SSR, so for each subject $SSR_{Intercept} \geq SSR_{Linear} \geq SSR_{Quadratic}$. In addition, for the by-subject regressions, the analyses are based on the criteria of minimizing the SSRs. In the random effects model, a subject's predicted value incorporates not only that subject's values, but is also influenced by the overall population data pattern, thereby attenuating extreme results. In addition to the 4 sets of by-subject SSRs, a subject's estimated intercept and slope as determined by the linear regression models method and the estimated intercept and slope as determined by the random effects model are provided in the electronic data set.

To further assess the results of the random effects model analysis, the renal event rate was estimated from the by-subject regression analysis. Following the same criterion as used for the random effects model, a subject was determined to have a renal event rate if the subject's slope from the by-subject linear regression was 0.143 or greater or 0.135 or greater corresponding to a serum creatinine increase of or 33% in 2-years or 50% in 3-years respectively.

Table 11-19 presents the estimated renal event rates based on the by-subject linear regression and the random effects model analyses.

Table 11-19: Estimated Renal Events Rates Based on By-Subject Regressions

	33% Increase Over 2-Year Criteria	50% Increase Over 3-Year Criteria
Linear Regression Renal Event Rate (# events / # subjects)	32% (27/85)	32% (27/85)
Quadratic Regression Renal Event Rate (# events / # subjects)	25% (16/64)	40.6 (26/64)

Note: a 33% Increase Over 2-Year Criteria corresponds to a subjects slope ≥ 0.143 , a 50% Increase Over 3-Year Criteria corresponds to a subjects slope ≥ 0.135

Reference: Table 14.8-9 and Table 14.8-15

As can be seen from Table 11-19, the renal event rates as estimated from the by-subject linear regression and the random effects model are similar. The comparability of these results lends support to the use of the random effects model in the estimation of renal event rates. In comparing the two methods, the random effects model has the advantage that it incorporates all of the available data. In addition, in the random effects model, the estimation of a patient's slope and intercept incorporates not only that patient's values, but is also influenced by the overall population data pattern, thereby decreasing the magnitude of extreme slope and intercept estimates.

11.5.4.8 Likelihood Analysis

The robustness of the renal event rate is investigated by estimating the mean likelihood that a patient will progress to a renal event in 3-years. The method for estimating renal event rates is to determine a dichotomous outcome (renal event, no renal event) for each patient based upon the estimated percent change in serum creatinine at 3-years as determined by the linear random effects model. This process is modified such that, as opposed to a dichotomous outcome, a continuous estimate of the patient's likelihood of progressing to a renal event is estimated.

For the 3-year 50% increase from baseline (i.e., Qualification Start Date) renal event rate criteria, the likelihood is calculated by estimating the subject's 3-year percent change from baseline, subtracting the 50% cut-point criteria in log-scale, and dividing by the corresponding standard error. Hence, if a patient's estimated 3-year percent change from baseline in serum creatinine is high, the likelihood of the patient progressing to a 50% increase within 3-years will be closer to 1 than a patient with a relatively low estimated percent change. The equation is as follows:

$$\text{Likelihood} = \Phi\{(3 \times \text{Slp} - \log(1.5)) / (3 \times \text{StdErr}(\text{Slp}))\}$$

where: *Slp* is the subject's estimated slope

log(1.5) is the 50% criteria in log-scale

StdErr(Slp) is the standard error of the subject's random slope effect.

Φ is the standard normal distribution.

The method is similar for the 2-year 33% change from baseline criteria.

The mean of the patient's likelihoods (which can be interpreted as an estimate of the renal event rate) is 34.9% based on the 50% criteria and is 33.6% based on the 33% criteria. This can be interpreted as an estimate of the renal event rate.

11.5.4.9 Subset Analyses

To assess the robustness of the renal event rate, subset analyses excluding patient's observations from the analyses were requested by the FDA. The exclusion criteria are as follows.

Exclusion Criteria 1

If a patient has a post-baseline (i.e., Qualification Start Date) serum creatinine value greater than 2.0 times the baseline value, exclude that observation and all subsequent observations for that subject.

Exclusion Criteria 2

If a patient has a serum creatinine value such that the value is an increase from the previous serum creatinine value of greater than 50% *and* the time that has elapsed between these 2 observations is greater than 1 year, exclude that observation and all subsequent observations.

Exclusion Criteria 3

Combine Criteria 1 and Criteria 2.

In addition to the method of excluding observations based upon the three criteria, supplemental analyses were performed where the criteria were applied but observations meeting the criteria were replaced with an upper limit value as opposed to excluding the observation. The rationale for the replacement methodology is that replacing as opposed to excluding the values provides a more accurate representation of the underlying population while still addressing restrictions imposed by the criteria. The details of the replacement criteria are as follows:

Replacement Criteria 1

If a subject has a post-baseline serum creatinine value greater than 2.0 times the baseline value, replace that observation with 2.0 times the subject's baseline serum creatinine value.

Replacement Criteria 2

If a subject has a serum creatinine value such that the value is an increase from the previous serum creatinine value of greater than 50% *and* the time that has elapsed between these 2 observations is greater than 1 year, replace that observation with 1.5 times the subject's previous observation.

Replacement Criteria 3

This method uses the minimum serum creatinine value from the Criteria 1 and Criteria 2 replacement methodologies.

There are concerns with these methods, the estimated renal event rates obtained by the random-effects model and the exclusion criteria are likely biased, since elimination of observations is based on the outcome. This is a non-ignorable pattern for missing data and the random-effects model is biased when missing data is non-ignorable.

The replacement methods adjust large measurements downward. These methods operate on outcome and are likely biased; however this bias should be less than the methods based on eliminating the measurement.

Table 11-20 and Table 11-21 list the estimated renal event rates based upon the random effects model with the subset exclusion and replacement observations.

Table 11-20: Estimated Renal Event Rate (50% Criteria) Based on the Linear Random Effects Model with Exclusion and Replacement Criteria

Estimated Renal Event Rates: 50% Increase Over 3-Years

	Estimated Renal Event Rate* (# of events / # of patients)	
	Exclusion of Observations	Replacement of Observations
Criteria 1	22 (23/ 104)	24 (25/ 104)
Criteria 2	26 (27/ 104)	31 (32/ 104)
Criteria 3	20 (21/ 104)	23 (24/ 104)

*The 50% increase over 3-years corresponds to a slope cut-point of 0.135
 Reference: Table 14.8-13

Table 11-21: Estimated Renal Event Rate (33% Criteria) Based on the Linear Random Effects Model with Exclusion and Replacement Criteria

Estimated Renal Event Rates: 33% Increase Over 2-Years

	Estimated Renal Event Rate* (# of events / # of patients)	
	Exclusion of Observations	Replacement of Observations
Criteria 1	20 (21/ 104)	24 (25/ 104)
Criteria 2	23 (24/ 104)	28 (29/ 104)
Criteria 3	18 (19/ 104)	23 (24/ 104)

*The 33% increase over 2-years corresponds to a slope cut-point of 0.143
 Reference: Table 14.8-13

There is a decrease in the estimated renal event rate based on the subset exclusion criteria populations. This is not surprising as elimination of observations is based on a patient having an increase in serum creatinine. When using the replacement methodology, the estimates of event rate also decrease, but by a smaller amount. The replacement methods adjust large measurements downward. These methods operate on outcome and are likely biased; however this bias should be less than the methods based on eliminating the measurement.

11.5.4.10 Analysis of 1 / Serum Creatinine

The reciprocal of serum creatinine (1/serum creatinine) was modeled as opposed to log (serum creatinine) as a method of assessing the robustness of the estimated renal event rate. The random effects model structure is analogous to the primary model, a linear fixed and random effects model, with the dependent variable being the reciprocal of serum creatinine as opposed to log (serum

creatinine). The criteria for a renal event are still an estimated 50% increase from baseline in serum creatinine within 3-years and a 33% increase from baseline in serum creatinine within 2-years.

Figure 11-22 presents the standardized residuals from the model versus quantiles of the standard normal distribution for both the logarithmic and reciprocal transformations. The figure illustrates that both transformations yield slightly more mass at zero than the standard normal distribution, but both transformations are reasonable.

Figure 11-22: Plot of Standardized Residuals for the 1/Creatinine Model versus Quantiles of Log Transformation and Reciprocal Transformation

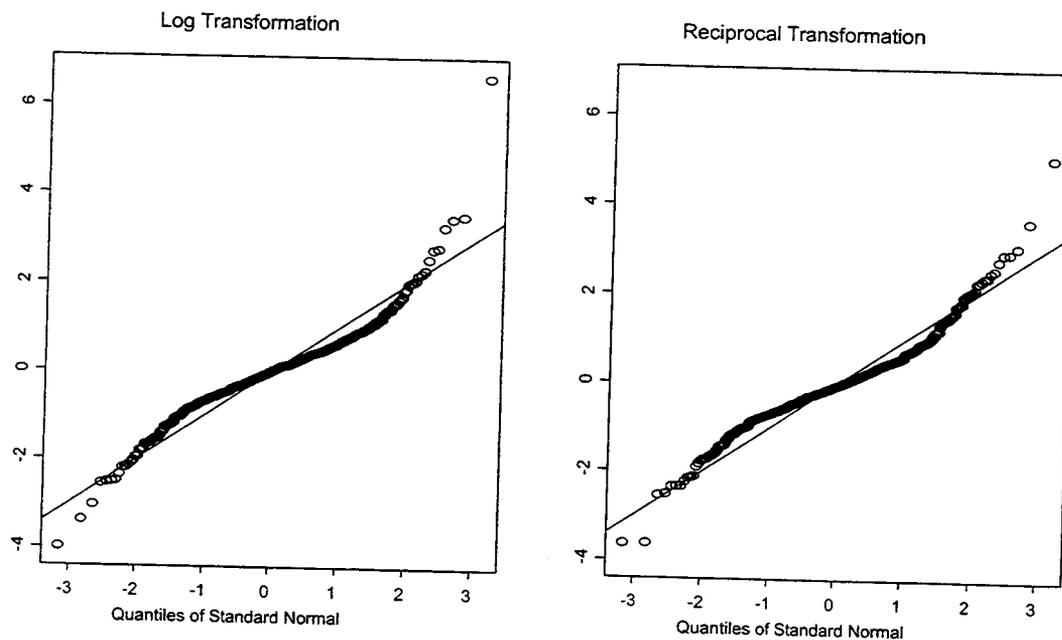


Table 11-22 compares the estimated renal event rates using the logarithmic versus reciprocal transformations under various subsets of the data.

Table 11-22: Estimated Renal Event Rates Using Logarithmic versus Reciprocal Transformations

Comparison Estimated Renal Event Rates: 50% Increase Over 3-Years

	Log Transformation	Reciprocal Transformation
Time of Observation	3 Year Event Rate (%)	3 Year Event Rate (%)
All Data	35	23
≥2 Obs	32	24
≥3 Obs	31	27
≤ 5 Years	32	26.0
Reference: Table 14.8-11; Table 14.8-12; Table 14.8-14; Table 14.8-18		

In summary, both the reciprocal and log transformations appear to satisfy the assumptions of the random-effects model. The renal event estimate based on the reciprocal transformation is somewhat lower than the estimated rate based on the log transformation. We chose to use the log transformation since it calibrates the data to percent change in serum creatinine.

11.5.4.11 Historical Predicted Glomerular Filtration Rate

The MDRD equation was used to estimate a patient’s Glomerular Filtration Rate (GFR) (see Section 9.7.2.1.3). Gender, race, serum creatinine value, and age at the serum creatinine value are taken into account in this equation (5 patients with missing ethnicity were categorized as non-black).

To further support the renal event rate as determined by the analysis of serum creatinine, GFR values were also modeled to estimate the renal event rate. The same random effects modeling methods used for the serum creatinine analyses were applied to the GFR values.

The patients’ slope cut-points for the estimated GFR are – 0.161 (corresponding to the 50% 3-year serum creatinine criterion) and – 0.170 (corresponding to the 33% 2-years serum creatinine criterion) were used. Patients with an estimated slope equal to or less than these cut-points were defined as having had an estimated renal event. Table 11-23 summarizes the availability of GFR records for AGAL-008-00 Qualified patients population and the Total population. The populations for the GFR analyses are identical to the corresponding serum creatinine analysis populations (see Table 11-10 and Table 11-24).

Table 11-23: Glomerular Filtration Rate (GFR) Records

GFR Records Available	Qualified Patients n (%)	All Patients n (%)
Total Patients	104/447 (23)	395/447 (88)
1 record	19 (18)	107 (27)
2 records	21 (20)	83 (21)
3 – 5 records	26 (25)	96 (24)
> 5 records	38 (37)	109 (28)
Reference: Table 14.9-2		

Table 11-24: Populations Analyzed in Event Rate Estimation

Patient Groups	n (%)
Qualified Patients (i.e., met Inclusion/Exclusion Criteria of AGAL-008-00)	104 (100)
with ≥ 2 Serial GFR Measurements	85 (82)
with ≥ 3 Serial GFR Measurements	64 (62)
Reference: Table 14.9-2, 14.9-3	

The results for the estimated event rate based on GFR are summarized in Table 11-25. The two- and three-year renal event rates derived from these models based on the estimated GFR values fall in the range of 28 – 34 %. These results are similar to those derived from the linear random effects model based on serum creatinine measurements.

Table 11-25: Estimated Event Rate for Qualified Population

	Number of Patients	Two Year Event Rate		Three Year Event Rate	
		Number With Slope ≤ 0.170	Event Rate %	Number With Slope ≤ 0.161	Event Rate %
All Qualified	104	32	31 (32/104)	35	34 (35/104)
≥ 2 observations	85	24	28 (24/85)	26	31 (26/85)
≥ 3 observations	64	19	30 (19/64)	21	33 (21/64)
Reference: Table 14.9-3					

Similar to the analysis of serum creatinine, covariate analysis was performed using the estimated GFR measurements. Table 11-26 summarizes the result of these analyses. The covariate parameter estimate, p-value, and the estimate of the renal event rate are presented.

Table 11-26: Parameter Estimates for Selected Covariates and Adjusted Renal Event Rate

Covariate Included	Covariate Description	Parameter Estimate	p-value	Adjusted 33% Renal Event Rate	Adjusted 50% Renal Event Rate
Age	Age at Qualification Date	-0.0092	0.005	28.85 (30/104)	30.77 (32/104)
Gender	Dichotomous: Male/Female	0.1076	0.477	29.81 (31/104)	32.69 (34/104)
Weight	Weight at Qualification Date	-0.0080	0.008	33.33 (29/87)	35.63 (31/87)
Blood Type	Dichotomous: {A+, A-, O+, O-} or {B+, B-, AB+, AB-}	0.1107	0.317	31.34 (21/67)	32.84 (22/67)
Plasma a-GAL	Plasma a-GAL level	0.0448	0.579	28.57 (16/56)	30.36 (17/56)
ACE Inhibitor Use	- Prior to Qualification Start Date - On or After Qual. Start Date - No ACE Inhibitor Data Recorded	-0.1033 0.1368	0.306 0.105	31.73 (33/104)	32.69 (34/104)

Renal event rate = (# estimated renal events/# patients) based on slope cut-point of -0.161 corresponding to a 50% increase in serum creatinine over a 3-year period and slope cut-point of -0.170 corresponding to a 33% increase in serum creatinine over a 2-year period.

Reference: Table 14.9-5

The estimated renal event rate analyses based on patients' GFR values were comparable to those based on serum creatinine. These results support the use of the random effects model as applied to the serum creatinine data.

11.5.4.12 Handling of Dropouts or Missing Data

Not Applicable

11.5.4.13 Interim Analyses and Data Monitoring

Two interim reports were submitted to FDA in April 2002 and July 2002.

11.5.4.14 Multicenter Studies

Not Applicable

11.5.4.15 Multiple Comparison/Multiplicity

Not Applicable

11.5.4.16 Use of an "Efficacy Subset" of Patients

Not Applicable

11.5.4.17 Active-Control Studies Intended to Show Equivalence

Not Applicable

11.5.4.18 Examination of Subgroups

Refer to Section 10.1.1 for definitions of subgroups analyzed.

11.5.5 Tabulation of Individual Response Data

Not Applicable

11.5.6 Drug Dose, Drug Concentration, and Relationships to Response

Not Applicable

11.5.7 Drug-Drug and Drug-Disease Interactions

Not Applicable

11.5.8 By-Patient Displays

Not Applicable

11.5.9 Application of Natural History Data to Phase 4 Data and Justification of the Sample Size for the Phase 4 Study (AGAL-008-00)

It is proposed that the estimated 3-year renal event rate from the natural history study and corresponding confidence interval be used as a comparison to the Phase 4 study. The renal event rate of the single arm Phase 4 Fabrazyme® study will be determined to be different from the natural history study if their corresponding 84% confidence intervals are non-overlapping.

For the historical control event rate, 80%, 84%, 90%, and 95% confidence intervals are presented based upon the confidence interval for the slope parameter in the linear random effects model. It should be noted that non-overlapping 84% Confidence Intervals for the individual renal event rate proportions are approximately equivalent to the corresponding 95% Confidence Interval for the difference of the parameters not including 0 (see Appendix 16.1.9).

For AGAL-008-00 treated patients, exact 80%, 84%, 90%, and 95% confidence intervals were calculated for various projected event rates (Table 11-27). The calculations were based on a historical control population of n=104 and on the projected enrollment of n=70 for the AGAL-008-00 study. Justification of sample size and power calculations for the Phase 4 study are provided in Appendix 16.1.9.

Table 11-27: A Comparison of the Estimated Three-Year Event Rates from Historical Control Population versus the Projected Three-Year Event Rates from r-hαGAL (AGAL-008-00) Treated Patients

Estimated 3-yr Event Rate (50% increase in serum creatinine) in Historical Database AGAL-008-00 Qualifiers (N=104)		Projected 3-yr Event Rate (50% increase in serum creatinine) in AGAL-008-00 Patients (N=70; final projected enrollment)		
Event Rate	32%	10%	15%	20%
80% Confidence Interval	25, 36	6, 16	10, 23	14, 27
84% Confidence Interval	25, 36	5, 17	10, 23	13, 28
90% Confidence Interval	24, 36	5, 18	9, 25	13, 29
95% Confidence Interval	24, 40	4, 20	8, 26	11, 31
The confidence interval for the historical control event rate is based on the confidence interval for the slope parameter in the linear random effects model. The confidence interval for the projected event rate is based on a two-tailed Fisher-Exact test. Reference: Table 14.8-6				

In addition to providing information on the power of the AGAL-008-00 Phase 4 study as a single arm historical control study, the natural history study provides information as to the accuracy of the renal event rate estimates used in the AGAL-008-00 double-blind placebo controlled power and sample size calculations. The estimated renal event rate from the historical study is 32%. Adjusting the 32% renal event rate for the disparity between the age of the historical study patients and the Phase 4 study patients (37.7 versus 45.8 respectively) yields an event rate of approximately 37% which is close to the 40% placebo renal event rate used in the AGAL-008-00 placebo controlled double-blind power calculations. Therefore, this analysis result lends support to the initial power calculations, that a sample size of approximately 70 patients with total study duration of 35 months is adequate for the double-blind trial (AGAL-008-00).

11.5.10 Efficacy Conclusions

The results from the historical database are reasonably robust to use the estimated 3 year renal event rate (as determined by a 50% increase in serum creatinine) as a basis for comparison to an amended version of the ongoing AGAL-008-00 trial for establishing the efficacy of Fabrazyme®. In addition, the analyses performed on the data in the historical database to support conversion of the AGAL-008-00 trial to a historical control show that these analysis methods can also support the use of this data as a comparator for other ongoing and future clinical trials.

12. SAFETY EVALUATION

This section not applicable for this report.

13. DISCUSSION AND OVERALL CONCLUSIONS

Based on the data derived from the AGAL-014-01 historical database and presented in this report, Genzyme proposes conversion of the current placebo-controlled Phase 4 study (AGAL-008-00) to an active treatment, historical control study design. In addition, based on the analysis of renal event rates using a linear trend random effects model, Genzyme would use the renal endpoint corresponding to a 3-year 50% increase from initial serum creatinine levels.

The natural logarithm of serum creatinine was modeled using a linear trend random effects. The criteria used to determine a renal event were an increase from Qualification Start Date in serum creatinine level of 33% within 2-years and 50% within 3-years. A subject was determined to have had a renal event if their estimated percent change from baseline (i.e., Qualification Start Date) in serum creatinine was above the 33% or 50% criteria.

Based on the linear trend random effects model, the estimated renal event rate is 30% for the 2-year 33% criteria and 32% for the 3-year 50% criteria for AGAL 008-00 Qualified patients. The estimation of the renal event rates based on the linear trend random effects model were shown to be robust by the following supplementary analyses: empirical estimation methods, exclusion of patients with limited number of observations, inclusion of covariates in the modeling, analysis of estimated GFR, and inclusion of quadratic trends in the model.

It is proposed that the estimated 3-year renal event rate from the natural history study and corresponding confidence interval be used as a comparison to the Phase 4 study. The renal event rate of the single arm Phase 4 Fabrazyme® study will be determined to be different from the natural history study if their corresponding 84% confidence intervals are non-overlapping.

Genzyme believes that the new proposed design can establish the clinical benefit of treatment with r-hαGAL and believes that the data from AGAL-014-01 is reasonably robust to support the proposal to convert the study to a single arm, active treatment, historical control design. Using this historical database as a comparator to the population treated with r-hαGAL in AGAL-008-00 would meet the definition of “an adequate, well-controlled study” pursuant 21 CFR 314.125. Further the justification for the proposed active treatment, historical control design is based on the ethical issue of maintaining a placebo-control arm as a study comparator while a suitable comparator population is available in the historical database.

DRAFT

Statistical Analysis Plan

**Assessing the Efficacy of Fabrazyme[®] in
a Phase 4 Study**

Prepared by: Donald B. Rubin, Ph.D.
John L. Loeb Professor of Statistics
Chairman, Dept. of Statistics
Harvard University
Cambridge, MA – 02138

With the assistance of Elizabeth A. Stuart, A.M., and Samantha R.
Cook, A.M., Department of Statistics, Harvard University

TABLE OF CONTENTS

1.	OBJECTIVE	2
2.	STUDY SUMMARIES	2
2.1	RANDOMIZED DOUBLE-BLIND STUDY.....	2
2.2	HISTORICAL CONTROL STUDY.....	3
3.	ANALYSIS METHODOLOGY SUMMARY	3
3.1	STAGE 1	4
3.1.1	OVERVIEW OF PROPENSITY SCORE MATCHING.....	4
3.1.2	SELECTING BASELINE FOR HISTORICAL CONTROLS.....	5
3.1.3	STEP 1--ESTIMATE PROPENSITY SCORES FOR HISTORICAL CONTROLS VERSUS RANDOMIZED.....	6
3.1.4	STEP 2--SELECT VERSION OF EACH HISTORICAL CONTROL.....	6
3.1.5	STEP 3--DISCARD THE IMPLAUSIBLE HISTORICAL CONTROLS.....	8
3.1.6	STEP 4--RE-ESTIMATE PROPENSITY SCORES IN RANDOMIZED VERSUS PLAUSIBLE HISTORICAL CONTROLS AND DISCARD.....	8
3.1.7	STEP 5--RE-ESTIMATE PROPENSITY SCORES IN RANDOMIZED AND CHOSEN HISTORICAL CONTROLS, AND SUBCLASIFY	8
3.2	STAGE 2.....	8
3.2.1	STEP 6--PATTERN OF HISTORICAL CONTROLS MISSING OUTCOMES.....	9
3.2.2	PLACEBO CONTROLS' MISSING OUTCOMES.....	9
3.2.3	STEP 7--BREAKING THE BLIND.....	10
3.2.4	STEP 8--REFINED PROPENSITY SCORE CLASSIFICATION.....	10
3.2.5	STEP 9--BUILD PREDICTION MODELS FOR DISEASE PROGRESSION WHEN UNTREATED.....	10
3.2.6	THE IMPUTATION MODEL	10
3.2.7	STEP 10--MULTIPLY-IMPUTE ALL SUBJECTS AS IF TREATED.....	11
3.3	STAGE 3	11
3.3.1	STEP 11--CALCULATE THE PRIMARY OUTCOME MEASURE FOR EACH TREATED PATIENT AS IF UNTREATED.....	11
3.3.2	STEP 12--COMPARE PREDICTIONS AS IF UNTREATED WITH ACTUAL OUTCOMES FOR THE TREATED.....	11
4.	REFERENCES	12
5.	APPENDIX	14

1. OBJECTIVE

The objective of this analysis will be to compare the renal event rate in a Fabrazyme[®] treated population to appropriate control groups. For this analysis a renal event involves the assessment of the increase from baseline in serum creatinine within three years. A renal event is defined as an increase of 50% in serum creatinine from baseline. Specifically, the objective will be to compare the treated patients in the phase 4 randomized double-blind AGAL-008-00 study to appropriate untreated controls. There are two sets of these controls. The first set is composed of the placebo controls from AGAL-008-00. The second set is an appropriately matched control group from the AGAL-014-01 historical control study.

2. STUDY SUMMARIES

The information in this section was provided to Datametrics Research Inc. by Genzyme, as were other details of the studies and measurements.

2.1 Randomized Double-Blind Study

AGAL-008-00 is an ongoing randomized double-blind placebo controlled study to assess the safety and efficacy of Fabrazyme[®]. As of November 18, 2002, 75 patients have been randomized using a 2:1 ratio of Fabrazyme[®] to placebo. The enrollment criteria for the study included that the patient must be at least 16 years old and have a serum creatinine level of 1.2 to 3.0 mg/dL or a serum creatinine value less than 1.2 mg/dL and an estimated creatinine clearance less than 80 mL/min. Women over 60 (n=3), although randomized, are excluded from all analyses because matching controls are not available. The primary efficacy objective of this study is to demonstrate the reduction in time to a clinical event in untreated patients as compared to Fabrazyme[®] patients. Whereas the placebo-controlled study focuses on a composite outcome of significant cardiac, renal, cerebral vascular events and/or death, the focus of the single-arm historical-control will be on renal events. After AGAL-008 becomes open label, it is desirable to modify how it is viewed because all patients will be on Fabrazyme[®], although those originally assigned to the treated group will have been exposed to treatment for a longer period of time than those originally assigned to placebo. One branch of the analysis will assess the efficacy of Fabrazyme[®] by comparing the renal event rate in the AGAL-008-00 originally treated group to the event rate in the placebo controls as if they had remained on placebo after the trial becomes open-label. Another analysis will involve comparing those initially assigned Fabrazyme[®] with an appropriate subset of the AGAL-014-01 historical study population. Thus, the control group for those randomized to Fabrazyme[®] will have two parts: those originally randomized to placebo plus the matched historical controls. A secondary analysis is within those initially assigned placebo, where the comparison is

between their actual outcomes post open-label and their projected outcomes as if they had remained on placebo. More detail on these analyses follow in Section 3.

2.2 Historical Control Study

AGAL-014-01 was an international, multicenter study of a historical cohort of patients with Fabry disease. The study had 27 participating sites collecting data on 447 patients with a current diagnosis of Fabry disease or who had a diagnosis of Fabry disease at the time of death. For AGAL-014-01, patients were required to meet each of the following criteria to be enrolled in this study:

1. current diagnosis of Fabry disease, or diagnosis of Fabry disease at time of death
2. patient, guardian, or next of kin must provide informed consent, including consent to release medical records.

Furthermore, the pool of possible patients was restricted to those who essentially met the randomized study criteria (age 16 or older, serum creatinine in a range approximately corresponding to the randomized trial range); there were 117 patients remaining in this pool.

3. ANALYSIS METHODOLOGY SUMMARY

Datametrics Research will perform the analysis of the data. Genzyme will be responsible for providing the necessary analysis data sets to Datametrics Research. The analysis will be done in three stages.

First, the historical control patients will be matched to the patients in the randomized study. The objective is to select a subset of the historical controls whose background characteristics plausibly could allow them to be in the randomized trial. The matching will be performed by Datametrics Research without any information corresponding to any patients' outcome data in either the historical data set or the randomized experiment. All implausible historical controls will be discarded. For this step, Datametrics Research will initially receive only the baseline characteristics of the patients. Five distinct steps are described in Section 3.1 for this stage.

The second stage will multipli-impute the missing data, both background and outcome, in the randomized experiment and in the plausible historical control data set in AGAL-008-00 as if no one had ever been treated with Fabrazyme[®]. At this stage, no data from those randomized to Fabrazyme[®] will be available to Datametrics Research. Five distinct steps are described in Section 3.2 for this stage.

Only after the first two stages are fully complete will the third stage begin: the analysis and comparison of outcome data from those randomized to Fabrazyme[®] with the outcome data predicted for them as if they had not been treated, based on the data from the plausible historical controls and those randomized to placebo. This stage will be the first

time any outcome data from the patients randomized to Fabrazyme® will be available to Datametrics Research.

3.1 Stage 1

3.1.1 Overview of Propensity Score Matching

The objective of matching the patients in the historical study to patients in the randomized study is to discard all historical controls who are not plausible points of comparison for the randomized group, selecting subjects based on covariates. This stage involves design and parallels the design of a randomized experiment. No outcome data are allowed at this stage; the only criteria involves balancing covariates. Thus, there is no opportunity to choose samples to obtain some result with respect to outcomes.

Propensity scoring algorithms (Rosenbaum and Rubin, 1983) following the method described in Rubin (2001) were utilized to select the historical patients who plausibly would have been in the randomized group. The end result is a subset of historical patients who resemble the randomized patients with respect to covariates. Table 3.1 lists the covariates considered in the generation of propensity scores. A combination of pair matching and sub classification was used (more details, below).

Table 3.1 List of Observed Covariates Used in the Matching Algorithm

- | | |
|---|---|
| • Baseline Serum Creatinine | • Baseline GFR ¹ |
| • Age | • Estimated Creatinine Clearance ² |
| • Gender | • Race |
| • Blood Group
{A+,A-,O+,O-}, {B+, B-,AB+, AB-} | • Plasma a-GAL |
| • Height | • Weight |

¹ GFR (ml/min/1.73m²) is estimated using the Modification of Diet in Renal Disease Study Group (MDRD) equation, which estimates GFR as a function of serum creatinine, age, gender, and race.

$GFR = 186 \times (\text{Serum Creatinine in mg/dL})^{-1.154} \times \text{Age}^{-0.203} \times (0.742, \text{ if patient is female}) \times (1.212, \text{ if patient is black})$

Levey AS, Greene T, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. Abstract. J Am Soc Nephrol. 11:2000.

² Creatinine clearance is estimated using the Cockcroft & Gault Formula

$\text{Creatinine clearance} = (0.85, \text{ if patient is female}) \times [(140 - \text{Age}) \times \text{Weight in kg}] / (72 \times \text{Serum Creatinine in mg/dL})$

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41

For some of these covariates, there are patients with missing values. The method described in D'Agostino and Rubin (2000) was used to estimate the propensity score, allowing for different patterns of missingness in the covariates. Based on a consideration of the likely reasons for missing data, it appears that it is reasonable to assume an ignorable missing data mechanism (i.e., missing at random, Rubin, 1976; Little and Rubin, 2002).

The general class of methods used to adjust for the covariates has appeared in a variety of publications, both theoretical (e.g., Rosenbaum and Rubin, 1983; Rubin and Thomas, 2000) and applied (e.g., Connors et al. 1996; Rubin, 1997). The specific approach used here combines aspects of matching and sub classification utilizing the propensity score.

To summarize briefly, the propensity score is defined by Rosenbaum and Rubin (1983) as the conditional probability of receiving a treatment given pre-treatment characteristics:

$$p(X_i) = \text{Prob.}\{W_i = 1 \mid X_i\}$$

where X_i is a vector of observed covariates, i indicates patients, and W_i is an indicator for received treatment. In a randomized trial, the propensity scores are known, whereas in an observational study, they must be estimated from the data on W_i and X_i . Of critical importance, no outcome data are required or desired to estimate, or to assess the success of the estimation of, the propensity score.

A principal theorem of propensity scores is that if a group of treated and control patients are matched relative to their propensity scores, differences between the two groups cannot be due to the observed covariates X_i . Hence, propensity scores are used to match groups of patients to reduce or eliminate biases in the data. As shown in a variety of places (e.g., Rosenbaum and Rubin, 1985; Rubin, 1979; Rubin and Thomas, 2000), Mahalanobis metric matching within propensity score calipers can be a beneficial refinement in many situations, and was partially used to obtain close matches on key covariates, as now discussed.

3.1.2 Selecting Baseline for Historical Controls

When designing an observational study such as this, which involves historical controls, it is important to try to replicate a randomized study design as closely as possible. Since the date that a historical control patient would have been entered into the study is not known, the baseline for a potential control patient is defined by the date that gives the closest covariate match to a randomized patient. Specifically, the set of potential matching patient observations was defined as any version of a historical control patient that met the eligibility criteria defined in Section 2.2 and that had at least one observation following it. This second criteria is parallel to the requirement in a randomized study that each patient agrees to at least one follow-up visit. There were 100 historical control patients that had at least one version eligible for matching, and a total of 395 possible versions for matching.

3.1.3 Step 1—Estimate Propensity Scores for Historical Controls vs. Randomized

The propensity score was estimated on this sample of 395 historical control observations and the 69 randomized patients whose covariate data were available at the time of this document. Due to the presence of missing covariates, the method described in D'Agostino and Rubin (2000) was utilized, as stated in Section 3.1.1. The specification included all variables listed in Table 3.1. Just the main effects were used in this step because the objective was to discard those historical controls clearly unlike the randomized group. The logistic transformation of the propensity score was used for the matching. The top sets of points in Figure 3.1 display the distribution of these propensity scores in these two groups. Table 3.2, Column 2, summarizes the balance of the covariates in the original sample of randomized patients and all versions of the historical controls.

3.1.4 Step 2—Select Version of Each Historical Control

For each historical control, select the same gender treated patient who is closest on covariates in the following sense: use Mahalanobis metric matching on age and baseline serum creatinine level within propensity score calipers.

1. For each version of a historical control patient, select all same gender randomized patients with a propensity score within 0.25 standard deviations of the historical control's propensity score.
2. Within this pool of close propensity score matches, select as a potential match the randomized patient with values of age and baseline serum creatinine closest to those of the historical control patient version (closest defined by the Mahalanobis distance).
3. Do step 2 for all versions of the historical control, and select the version with the smallest Mahalanobis distance to a randomized patient.
4. Define the chosen historical control's version as that historical patient's baseline, and eliminate all of that patient's other versions from the collection of potential historical matches.
5. Repeat steps 1-4 for each historical patient.

3.1.5 Step 3—Discard the Implausible Historical Controls

If there were no same gender randomized patients within the propensity score caliper for any version of a historical control, that historical control patient was considered implausible for the randomized experiment and so discarded. There were 10 historical control patients discarded by this process. There are 90 plausible historical controls. The third and fourth sets of points in Figure 3.2 show the propensity scores for the plausible

Table 3.2: Balance of Covariates in Matched Samples¹

	Randomized Group Mean (Standard Deviation)	Standardized Bias ² -- all versions of all Historical Controls	Standardized Bias ² -- Selected Versions of Historical Controls	Standardized Bias ² -- Plausible Controls	Standardized Bias ² -- Chosen Controls	Standardized Bias ² -- Chosen Controls (Subclass Estimates)
N	69	395	100	90	85	85
Sex	1.07 (0.26)	0.15	0.09	0.19	0.19	0.10
Ethnicity	1.91 (0.28)	0.11	0.19	0.16	0.07	-0.09
Blood Group	1.12 (0.33)	0.04	-0.08	-0.03	0.04	0.52
Age	45.1 (8.89)	0.56	0.89	0.75	0.72	-0.11
Weight	70.25 (12.44)	-0.27	-0.19	-0.13	-0.06	-0.21
Height	172.06 (15.35)	-0.20	-0.11	-0.07	-0.07	-0.03
Serum creatinine mg/dL	1.66 (0.52)	0.13	0.51	0.45	0.42	-0.06
Estimated GFR	52.70 (18.02)	-0.30	-0.73	-0.64	-0.61	0.04
Estimated creatinine clearance	59.41 (17.51)	-0.67	-1.08	-0.88	-0.77	-0.09
Plasma α - GAL	1.21 (1.02)	0.40	0.42	0.37	0.35	0.46
Linear Propensity Score ³	0 (1)	0.67	0.85	1.07	0.97	0.04

¹ Calculated using available case means, which can be especially deceptive in small samples.

² Standardized bias is defined the difference in means divided by the standard deviation in the randomized group.

³ Propensity score defined with regard to the column.

historical controls as well as the ten implausible historical controls (using their largest propensity score). The third column of Table 3.2 displays the balance between the randomized group and all single version historical controls. The data for the discarded historical controls are displayed in Appendix Table A-1.

3.1.6 Step 4—Re-estimate Propensity Scores in Randomized versus Plausible Historical Controls and Discard

Each plausible historical control has one version, and so we can re-estimate the propensity score for the 69 randomized patients versus these 90 plausible historical controls. The results are displayed in Figure 3.2 and column 4 of Table 3.2. Notice the left tail of the distribution of historical control propensity scores, with values quite dissimilar from any randomized patient. The five historical controls with propensity scores clearly lower than the minimum propensity score for a randomized patient are discarded, leaving 85 chosen historical controls within the range of the randomized group. The data for the five discarded historical controls are displayed in Appendix Table A-2.

3.1.7 Step 5—Re-estimate Propensity Scores in Randomized and Chosen Historical Controls, and Subclassify

Using the 85 chosen historical controls and the 69 randomized patients, re-estimate the propensity score. Based on these re-estimated propensity scores, form subclasses of approximately equal size in the randomized group. Because of the dearth of historical controls with large propensity scores, the top subclass had to be nearly twice the size of the other subclasses: the sizes are 12, 11, 12, 11, 23 randomized patients and 39, 27, 8, 7, 4 historical control patients. Results are displayed in Figure 3.3 and in Columns 5 and 6 of Table 3.2, Column 5 without sub classification, Column 6 using sub classification.

We do not expect to be using exactly these subclasses for our final analysis, but rather the subclasses created in Step 8.

3.2 Stage 2

The second stage in the data analysis plan to be conducted by Datametrics Research, Inc. is the imputation of missing outcome data (e.g., serum creatinine levels) for all patients in the study as if they had not been treated with Fabrazyme[®]. This imputation will be done multipli to allow all relevant sources of uncertainty to be reflected in all subsequent data analyses (Rubin, 1987; 1996).

In the randomized group, after baseline, laboratory measurements were taken every month within a window of plus or minus three days for three years. In the historical controls, these measurements were not taken with the same regularity, and the monthly lab measurement values are therefore often "missing". In the placebo controls, the missing outcomes are the values of serum creatinine when on placebo after the

randomized trial becomes open label, which has not occurred yet. At no point in Stage 2 will any outcome data from those treated with Fabrazyme[®] be available to Datametrics.

3.2.1 Step 6 – Pattern of Historical Controls’ Missing Outcomes

Figures 3.4 and 3.5 display the pattern of missing outcome data for the 85 historical controls chosen according to the analysis in Section 3.1. The horizontal axes are time, the 85 horizontal lines represent the 85 chosen historical controls, and the points represent the times of their observed data values. The time axis in Figure 3.3 is from minus two years from baseline to plus five years from baseline. In Figure 3.4, the time axis is from baseline to three years from baseline, and the vertical bands are every month, and are plus or minus three days, thereby representing the measurement time in the randomized group. Evidently, there are many historical controls with very little data, some with a lot of data, including extending beyond three years past baseline. It is important to emphasize, that at this point, Datametrics has intentionally not had access to any outcome data for either the randomized group or the historical controls. The pattern of missing outcome data shown in Figures 3.4 and 3.5 (and Table A-3 in the Appendix) reveal the difficulty of imputing the missing data, unless there is strong scientific knowledge about the progression of serum creatinine levels in untreated patients. For this reason, our plan is to utilize the data from the placebo controls when on placebo to inform this modeling and imputation. The long term data from the historical controls are informative about the progression of serum creatinine levels under no treatment for the time period after the randomized trial becomes open label.

3.2.2 Placebo Controls’ Missing Outcomes

The missing outcomes for the placebo controls are their serum creatinine levels if they had remained on placebo through the end of the trial, rather than switching to Fabrazyme[®] when the trial becomes open label. If we were to rely only on the data in the placebo group to conduct this imputation, it would be relatively speculative for two reasons. First, because of the randomization scheme, we expect to see only about 25 placebo controls once the blind is broken, a relatively limited sample. Second, since essentially all the missing data will occur after the trial is open-label, the imputation could involve almost entirely extrapolation, for example from trends seen in the first two years of data for the placebo controls when on placebo, to the third year when they are no longer on placebo. But this extrapolation of serum creatinine levels when untreated can be informed by the extended measurements of the historical controls, as displayed in Figures 3.4 and 3.5. Thus, the imputation of each of the control groups is greatly helped by the existence of outcome data in the other control group: the placebo controls provide detailed measurements of serum creatinine progression in the absence of treatment in the short term, whereas the historical controls provide measurements of the longer term progression in a larger group.

3.2.3 Step 7 – Breaking the Blind

In order to proceed, either with the refined propensity score sub classification described in Step 8 below, or doing the modeling of the outcomes in the placebo group implied in Section 3.2.2, we need to break the blind and know who is a randomized treated and who is a randomized placebo control. We still do not want to see any outcome data at this point. We now assume the blind is broken, at least to Datametrics Research, Inc., or to a third party.

3.2.4 Step 8 – Refined Propensity Score Classification

The propensity score between the treated group and the 85 chosen historical controls, and the propensity score between the placebo controls and the historical controls, are only randomly different from each other because the treated and placebo controls are a random split with respect to background covariates. This should be checked and verified. Datametrics Research, Inc., plans to do the relevant diagnostic analysis of the distribution of the propensity scores in these three separate groups: treated, placebo control, historical control. This will reveal the overlap in distribution between treated and control (placebo or historical). Using a possibly reestimated propensity score, Datametrics Research, Inc., plans to form new subclasses, where the hope is to have roughly equal-sized subclasses in the treated group (e.g., 5 subclasses of 10 each).

3.2.5 Step 9 – Build Prediction Models for Disease Progression when Untreated

In order to do the modeling of post-randomization disease progression when not treated, we need access to the outcome data for the chosen historical controls and for the placebo controls. But there shall be no access to the outcome data from the treated group. In fact, there should be no access to the outcome data from either control group until the specific forms of these prediction models are agreed upon. The outcomes for those randomized to treatment should not be used at any time in this stage.

Datametrics need not perform the analyses of the historical control data and the placebo control data, but the analyses must follow the guidelines in Rubin (1987) and Little and Rubin (2002) for proper multiple imputation. A third party could possibly do this once a model has been settled upon. Once selected, the model should be fit to the partially observed outcome data for the placebo controls and the chosen historical controls.

3.2.6 The imputation model

The choice of a scientifically defensible model to be used for the imputation of the missing outcomes for the historical controls and for the placebo controls is very important. Before the blind is broken, an acceptable model should be defined. It should be scientifically defensible and allow for all anticipated trends in serum creatinine levels, and should allow these trends to depend on important covariates, such as age, sex, baseline serum creatinine, etc., and propensity subclass. Datametrics does not have any

particular scientific expertise on the class of such pharmacological time series models, but can offer guidance on the aspects of the chosen model that make it most appropriate for objective multiple imputation. It is imperative for the objectivity of this endeavor, however, that FDA and Genzyme agree on a model before any outcome data from the randomized trial are made available.

3.2.7 Step 10 – Multiply-Impute All Subjects as if Untreated

Using the model chosen in Section 3.2.6 and estimated from the placebo and historical control outcome data, multiply impute missing outcome data, as if untreated, for all subjects. Notice, that this requires the covariates, including subclass, for the patients, including the treated, but still no outcome data for the treated.

3.3 Stage 3

At the end of the second stage, we will have a Fabrazyme[®] treated group of approximately 50, a chosen historical control group of approximately 85, and a placebo control group of approximately 25. Each set of multiple imputations will create one "complete" data set, with serum creatinine levels under Fabrazyme[®] for the treated and corresponding imputed and actual serum creatinine levels for the controls. Each such data set will be analyzed as specified, and the results combined using the standard multiple imputation combining rules to generate one inference for each analysis.

3.3.1 Step 11 – Calculate the Primary Outcome Measure for Each Treated Patient as if Untreated

For example, in each of the multiply imputed data sets, calculate the predicted number of events in the treated group if they were untreated, and thereby find a distribution of that number of events that reflects sampling variability – use this to form an interval estimate or hypothesis test. Or calculate the time that each patient is predicted to be event-free when untreated, or the number of events a patient is predicted to have when untreated, or K-M type statistic, etc. Again, it is critical that Genzyme and FDA agree on this before this step; ideally before step 9. Still no outcome data from the treated are being seen.

3.3.2 Step 12 – Compare Predictions as if Untreated with Actual Outcomes for the Treated

Finally, examine the outcome data from the treated. Compare the actual outcome data from the treated to the prediction intervals in step 11. If the real data fall below the predicted 95% interval, e.g., for number of events, the treatment works significantly better than no treatment.

4. REFERENCES

1. Connors, A.F. Jr., Speroff, T., Dawson, N.V., Thomas, C., Harrell, F.E. Jr., Wagner, D., et al. "The effectiveness of right heart catheterization in the initial care of critically ill patients." SUPPORT Investigators. *Journal of the American Medical Association* 276: 889-897, 1996.
2. D'Agostino, R. and Rubin, D.B. "Estimation and use of propensity scores with incomplete data." *Journal of the American Statistical Association* 95: 749-759, 2000.
3. Little, R., Rubin, D., *Statistical Analysis with Missing Data, 2nd Edition*. Wiley, New York, 2002.
4. Rosenbaum, P.R., and Rubin, D. "Constructing a control group using multivariate matched sampling methods that incorporate the propensity score." *American Statistician* 39: 33-38, 1985.
5. Rosenbaum, P.R., and Rubin, D. "The central role of the propensity score in observational studies for causal effects." *Biometrika* 70: 41-55, 1983.
6. Rubin, D., and Thomas, N., "Combining propensity score matching with additional adjustments for prognostic covariates." *Journal of the American Statistical Association* 95(450): 573-585, 2000.
7. Rubin, D., "Using Propensity Scores to Help Design Observational Studies: Application to the Tobacco Litigation". *Health Services & Outcomes Research Methodology* 2:169-188, 2001.
8. Rubin, D., "Estimating causal effects from large data sets using propensity scores." *Annals of Internal Medicine* 127: 757-763, 1997.
9. Rubin, D. "Multiple Imputation after 18+ years" (with discussion). *Journal of the American Statistical Association* 91: 473-489, 1996.
10. Rubin, D. *Multiple Imputation for non-response in surveys*. Wiley, New York, 1987.
11. Rubin, D. "Using multivariate matched sampling and regression adjustment to control bias in observational studies." *Journal of the American Statistical Association* 74: 318-328, 1979.

12. Rubin, D., "Inference and missing data." *Biometrika* 63: 581-590, 1976.

APPENDIX

Table A-1: Historical Controls with no Plausible Versions

Patient ID	Sex	Age	Serum Creatinine	Est, GFR	Est. creatinine clearance	Plasma α -GAL	Weight	Height	Ethnic ¹	Blood Group ²
6016	M	16	1.21	84.2	NA	NA	NA	NA	2	1
9024	F	17	0.89	88.4	78.5	NA	48.3	NA	1	NA
9024	F	21	0.87	87.2	75.8	NA	47.0	NA	1	NA
9024	F	33	0.92	74.9	68.7	NA	50.0	155	1	NA
16001	M	21	1.30	74.2	92.7	0.10	72.8	NA	2	2
20006	M	19	1.20	82.9	106.8	0.10	76.3	180.3	2	2
20006	M	19	1.10	91.7	116.5	0.10	76.3	180.3	2	2
20037	M	27	1.30	70.5	100.3	0.50	83.0	189.1	2	1
20037	M	27	1.20	77.3	108.7	0.50	83.0	189.1	2	1
20037	M	28	1.20	76.4	114.8	0.50	88.9	188.4	2	1
20037	M	32	1.10	82.3	126.9	0.50	93.3	185.0	2	1
20100	M	35	1.20	73.4	102.8	0.18	84.4	NA	2	NA
20100	M	35	1.10	81.0	112.0	0.18	84.4	NA	2	NA
20100	M	35	1.10	80.8	111.5	0.18	84.4	NA	2	NA
20100	M	35	1.10	80.8	111.4	0.18	84.4	172.7	2	NA
32045	F	34	1.10	60.6	128.5	1.49	112.5	NA	2	NA
47003	M	16	1.20	85.4	107.9	0.14	75.4	NA	2	NA
57081	M	31	1.20	74.9	99.6	NA	79.2	NA	1	1
57081	M	32	1.20	74.5	121.0	NA	97.0	NA	1	1
59007	F	32	0.88	78.8	68.7	NA	47.7	NA	2	1

¹ Blood Group 1 = "A+, A-, O+, O-", Blood Group 2 = "B+, B-, AB+, AB-"

² Ethnicity 1 = "Caucasian", Ethnicity 2 = "Non-Caucasian"

Table A-2: Historical Controls Out of Randomized Range

Patient ID	Sex	Age	Serum Creatinine	Est, GFR	Est. creatinine clearance	Plasma α -GAL	Weight	Height	Ethnic ¹	Blood Group ²
6022	M	34	1.14	77.9	117.3	NA	91.0	NA	2	2
9001	M	30	1.29	69.4	95.1	NA	80.5	NA	1	1
20008	M	37	1.30	65.9	81.1	0.00	73.8	NA	1	2
27003	M	35	1.10	81.2	123.6	1.49	92.8	NA	2	1
32029	M	34	1.20	73.8	92.7	0.00	75.3	NA	1	1

¹ Blood Group 1 = "A+, A-, O+, O-", Blood Group 2 = "B+, B-, AB+, AB-"

² Ethnicity 1 = "Caucasian", Ethnicity 2 = "Non-Caucasian"

Table A-3: Observations available for chosen historical controls

Patient ID	Number of observations	Number of observations within 3 years after baseline	Number of observations within 3 day windows	Number of observations within unique 3 day windows
6001	6	4	1	1
6006	9	7	2	2
6013	2	1	1	1
9003	3	1	1	1
9006	9	5	1	1
9007	4	3	3	3
9010	10	2	2	2
9021	3	3	1	1
13001	8	2	1	1
13003	13	4	2	2

Patient ID	Number of observations	Number of observations within 3 years after baseline	Number of observations within 3 day windows	Number of observations within unique 3 day windows
20004	4	2	2	2
20011	6	3	1	1
20014	2	2	1	1
20017	2	2	2	1
20019	3	2	2	1
20029	5	3	2	2
20035	5	2	1	1
20036	2	2	2	2
20041	2	2	1	1
20048	2	2	2	2
20075	4	3	1	1
20087	28	9	3	3
20094	2	2	1	1
20102	10	7	1	1
20112	2	2	2	2
20122	18	1	1	1
20124	2	2	1	1
21001	18	9	3	3
21004	5	5	2	2
25001	10	1	1	1
25005	8	6	4	4
27002	15	2	1	1
27004	15	10	3	3
27008	5	2	1	1

Patient ID	Number of observations	Number of observations within 3 years after baseline	Number of observations within 3 day windows	Number of observations within unique 3 day windows
27012	3	2	1	1
27013	19	6	4	1
27021	3	2	1	1
30001	2	2	1	1
30002	15	1	1	1
30003	2	2	1	1
32001	4	3	2	1
32002	11	2	1	1
32003	7	3	1	1
32030	3	2	1	1
32034	10	5	1	1
32039	12	2	2	2
32044	8	2	1	1
32047	13	3	1	1
34001	6	5	1	1
34003	5	1	1	1
34005	7	6	1	1
34006	8	2	1	1
35001	6	6	3	3
41002	2	1	1	1
48007	13	4	2	2
48012	2	2	1	1
57001	85	31	7	6
57002	4	2	1	1

Patient ID	Number of observations	Number of observations within 3 years after baseline	Number of observations within 3 day windows	Number of observations within unique 3 day windows
57003	9	3	3	3
57006	9	2	2	2
57009	2	2	1	1
57019	27	23	7	7
57024	15	4	1	1
57027	17	7	3	3
57037	13	9	1	1
57041	9	4	1	1
57046	34	10	3	2
57047	8	4	1	1
57050	7	6	1	1
57052	21	2	1	1
57053	8	5	4	3
57065	8	4	1	1
57070	8	2	1	1
57073	22	6	1	1
57074	26	16	4	3
57075	2	1	1	1
57077	6	4	1	1
57078	5	4	2	1
57080	8	1	1	1
57082	13	3	1	1
57084	4	2	2	2
57085	6	5	2	2

Patient ID	Number of observations	Number of observations within 3 years after baseline	Number of observations within 3 day windows	Number of observations within unique 3 day windows
57087	6	2	2	1
57091	9	2	2	1
57144	5	2	1	1

Figure 3.1: Propensity scores in randomized and multi version historical controls

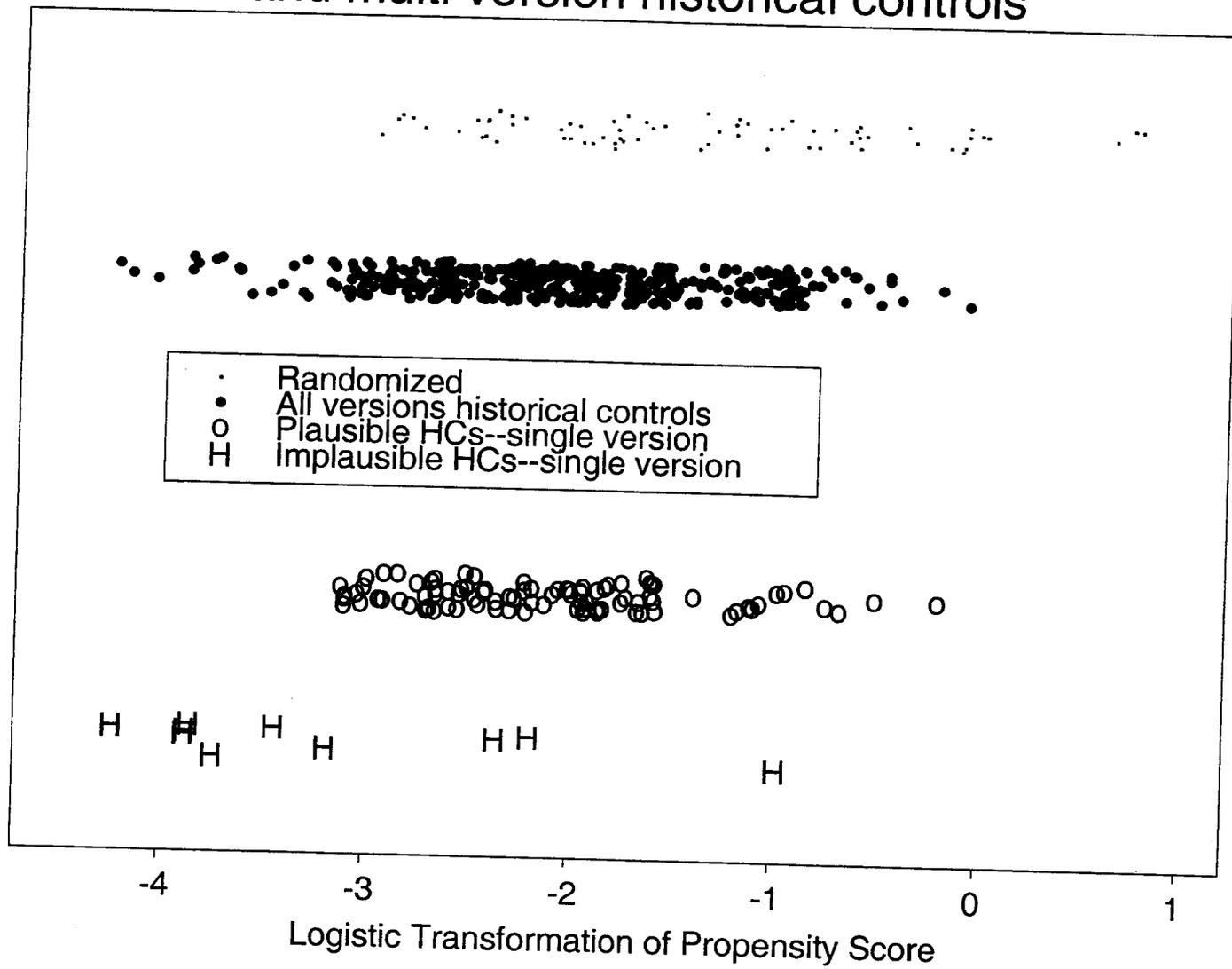


Figure 3.2: Propensity scores in randomized and plausible controls

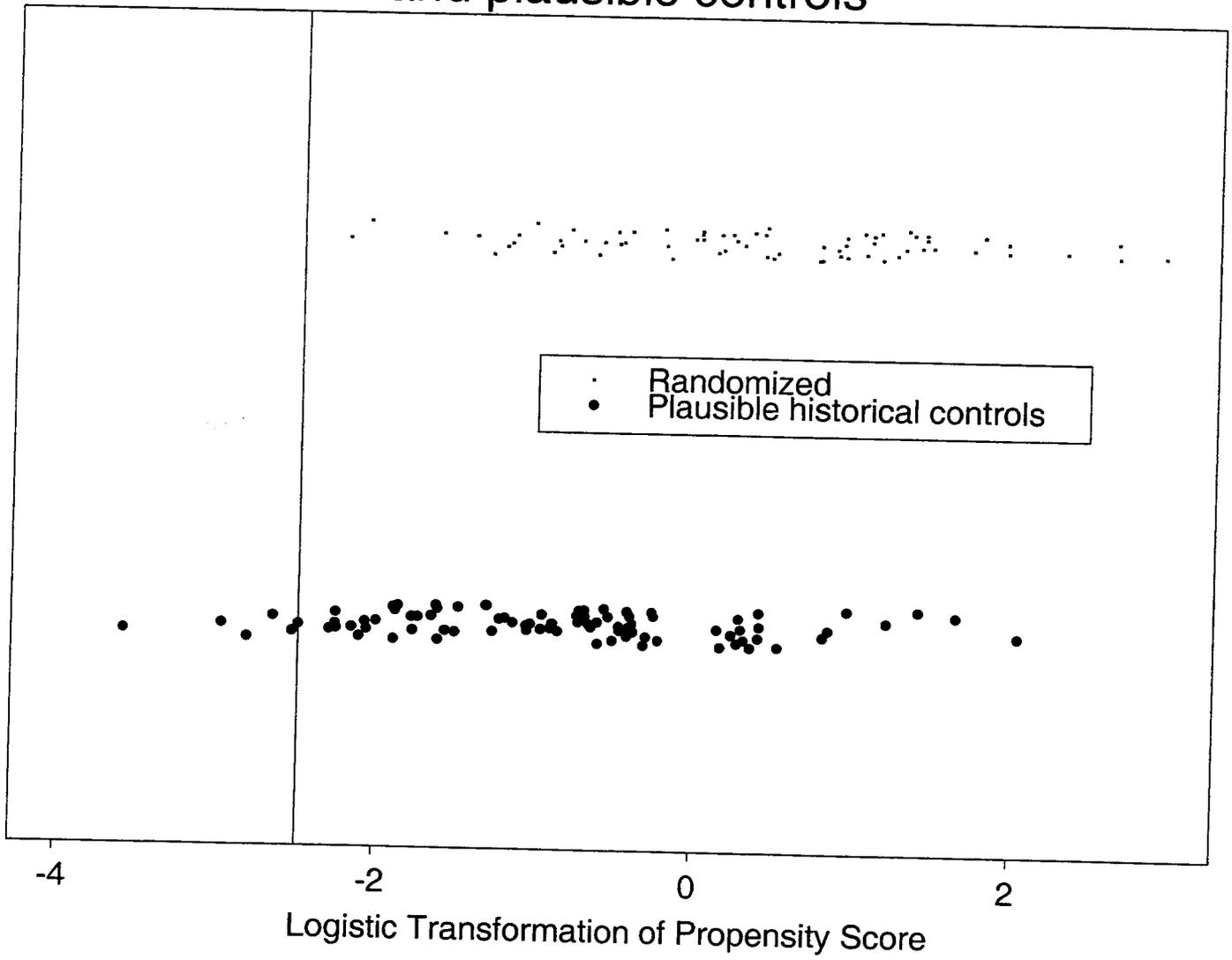


Figure 3.3: Propensity scores and subclassification in randomized and chosen historical controls

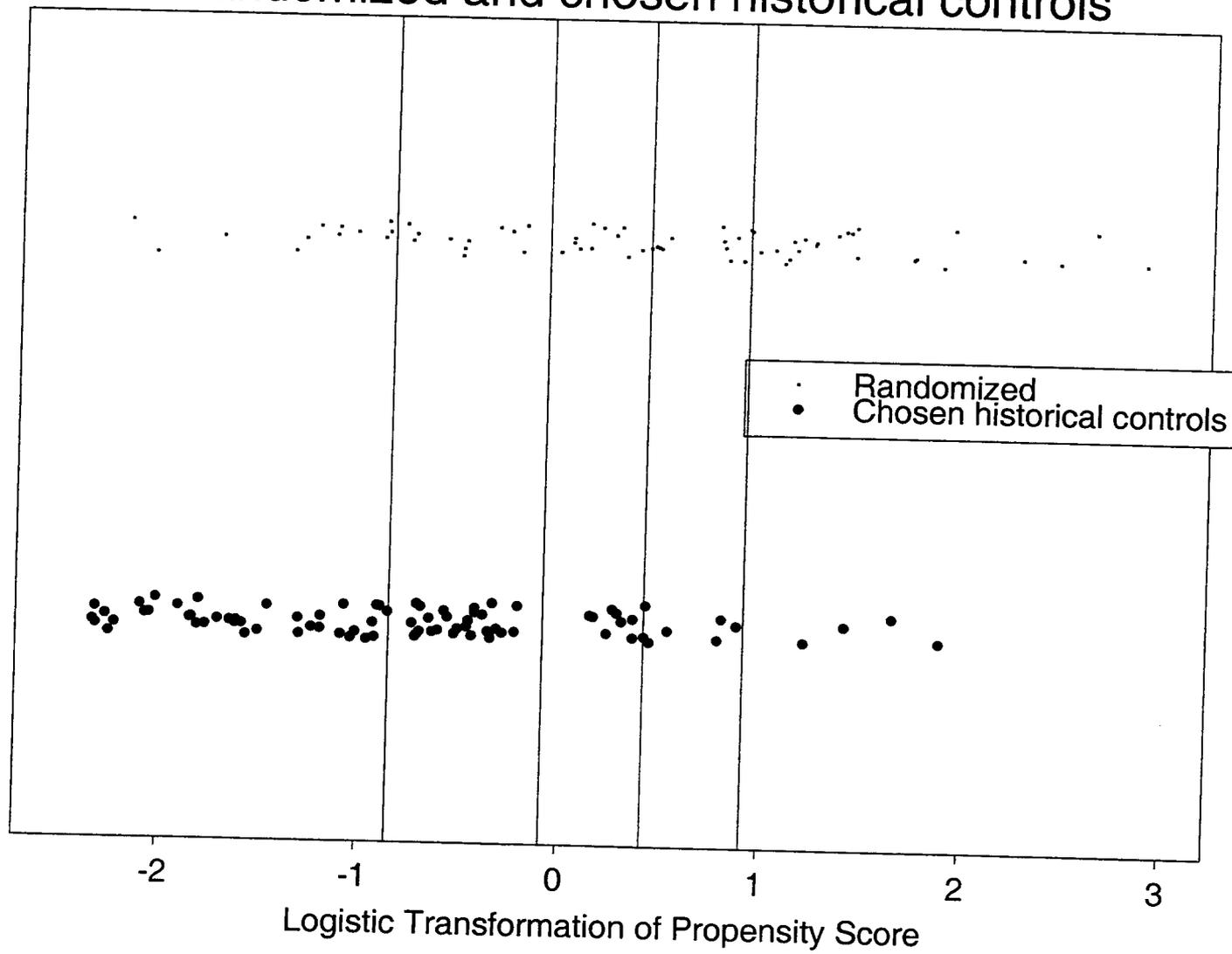


Figure 3.4

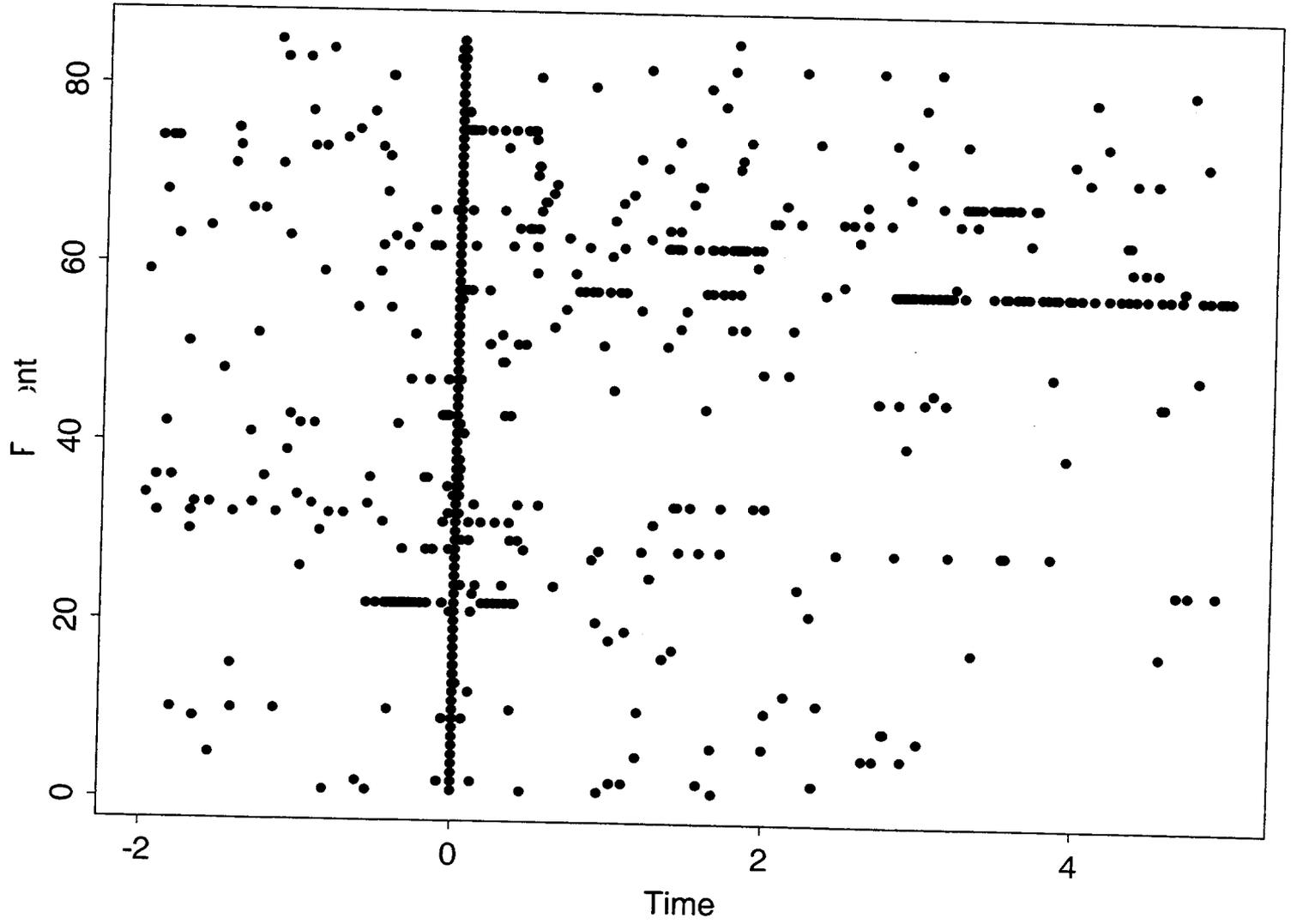
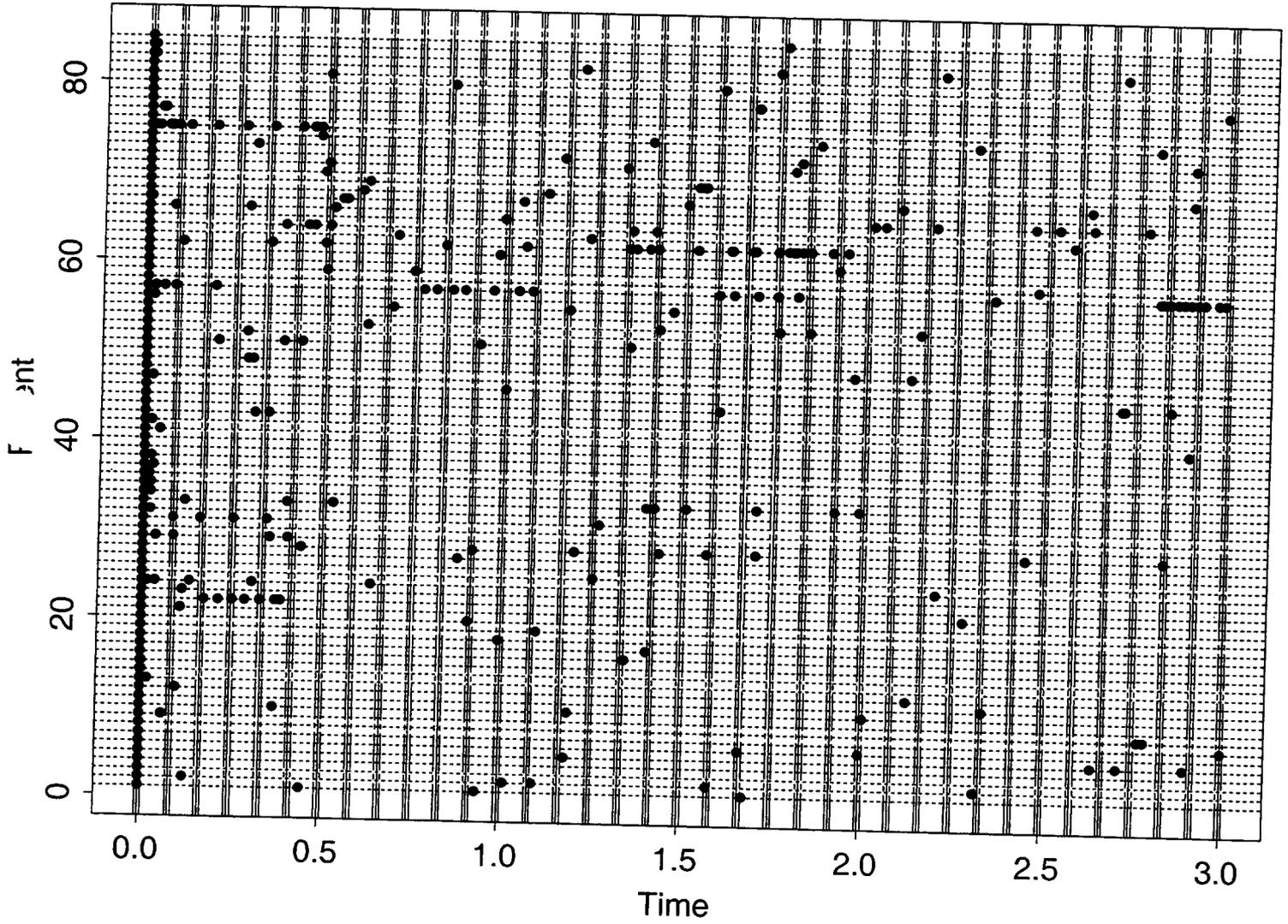


Figure 3.5



9. SELECTED REFERENCES

- Bass JL, Shrivastava S, Grabowski GA, Desnick RJ, Moller JH. The M-mode echocardiogram in Fabry's disease. *American Heart Journal*. 1980;100(6 Pt 1):807-812.
- Branton M, Schiffman R, Sarnis SG, Murray GJ, Quirk JM, et al. Influence of α -Galactosidase A activity and genetic mutations on clinical course. *Medicine*. 2002; 81(2):122-138.
- Brenner BM, Meyer TW, Hostetter TH. Dietary Protein Intake and the Progressive Nature of Kidney Disease: The Role of Hemodynamically Mediated Glomerular Injury in the Pathogenesis of Progressive Glomerular Sclerosis in Aging, Renal Ablation, and Intrinsic Renal Disease. *New England Journal of Medicine*. 1982; 307(11):652-659.
- Brenner BM. Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney International*. 1983; 23:647-655.
- Crutchfield KE, Patronas NJ, Dambrosia SM, Frei KP, Banerjee TK, et al. Quantitative analysis of cerebral vasculopathy in patients with Fabry disease. *Neurology*. 1998;50(6):1746-1749.
- DeGraba T, Azhar S, Dignac-George F, Brown E, Boutihre B, et al. Profile of Endothelial and Leukocyte Activation in Fabry Patients. *Annals of Neurology*. 2000; 47(2):229-233.
- Desnick RJ, Ioannou YA, Eng CM. α -Galactosidase A deficiency: Fabry disease. *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York; 1995:2741-2784.
- Desnick RJ. Enzyme Replacement and Beyond. *Journal of Inherited Metabolic Disease*. 2001:251-265.
- Elleder M, Bradova V, Smid F, Budesinsky M, Harzer K, et al. Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease. *Virchows Archiv A Pathological Anatomy and Histopathology*. 1990; 417:449-455.
- Eng C, Banikazemi M, Gordon R, Goldman M, Phelps R, et al. A Phase 1/2 Clinical Trial of Enzyme Replacement in Fabry Disease: Pharmacokinetic, Substrate Clearance, and Safety Studies. *American Journal of Human Genetics*. 2001; 68:711-722.
- Eng C, Guffon N, Wilcox W, Germain D, Lee P, et al. Safety and Efficacy of Recombinant Human α -Galactosidase A Replacement Therapy in Fabry's Disease. *New England Journal of Medicine*. 2001; 345(1):9-16.
- Farge D, Nadler S, Wolfe L, Barre P, Jothy S. Diagnostic Value of Kidney Biopsy in Heterozygous Fabry's Disease. *Archives of Pathology and Laboratory Medicine*. 1985; 109: 85-88.
- Ferrans VJ, Hibbs RG, Burda CD. The heart in Fabry's disease. A histochemical and electron microscope study. *American Journal of Cardiology*. 1969; 24(1):95-110.

- Filling-Katz MR, Merrick HF, Fink JK, Miles RB, Sokol J, Barton NW. Carbamazepine in Fabry's disease: effective analgesia with dose-dependent exacerbation of autonomic dysfunction. *Neurology*. 1989;39(4):598-600.
- Goldman ME, Cantor R, Schwartz MF, Baker M, Desnick RJ. Echocardiographic abnormalities and disease severity in Fabry's disease. *Journal of the American College of Cardiology*. 1986;7(5):1157-1161.
- Gordon KE, Ludman MD, Finley GA. Successful treatment of painful crises of Fabry disease with low dose morphine. *Pediatric Neurology*. 1995;12(3):250-251.
- Grewal RP. Stroke in Fabry's disease. *Journal of Neurology*. 1994; 2:153-156.
- Grunfeld JP, Lidove O, Barbey F. Heterozygotes with Fabry's Disease. *Contributions to Nephrology*. 2001; 136:208-210.
- Gubler MC, Lenoir G, Grunfeld JP, Ulmann A, Droz D, Habib R. Early renal changes in hemizygous and heterozygous patients with Fabry's disease. *Kidney International*. 1978; 13(3):223-235.
- Hasholt L, Sorenson SA. Lysosomal alpha-galactosidase in endothelial cell cultures established from a Fabry hemizygous and normal umbilical veins. *Human Genetics*. 1986; 72:72-6.
- Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *American Physiological Society*. 1981: 85-93.
- Ikari Y, Kuwako K, Yamaguchi T. Fabry's disease with complete atrioventricular block: histological evidence of involvement of the conduction system. *British Heart Journal*. 1992; 68:323-325.
- Inagaki M, Ohno K, Hisatome I, Tanaka Y, Takeshita K. Relative hypoxia of the extremities in Fabry disease. *Brain and Development*. 1992;14 (5):328-333.
- Ioannou Y, Zeidner K, Gordon R, Desnick R. Fabry Disease: Preclinical Studies Demonstrate the Effectiveness of α -Galactosidase A Replacement in Enzyme-Deficient Mice. *American Journal of Human Genetics*. 2001; 68:14-25.
- Junsanto T, Meehan SM, Rydell JJ, Desnick RJ. 2001. Fabry Disease: Proteinuria and podocyte pathology in a septuagenarian cardiac variant. Submitted to *Kidney International*.
- Laird NM and Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982;38:963-74.
- Lawlor GJ, Fisher TJ. *Manual of Allergy and Immunology*. Little, Brown and Company, Boston; 1988.

- Lenoir G, Rivron M, Gubler MC, Dufier JL, Tome FS, Guivarch M. Fabry's disease. Carbamazepinetherapy in acrodyniform syndrome. *Arch Fr Pediatr.* 1977;34(8):704-716.
- Levey AS, Greene T, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. Abstract. *Journal of the American Society of Nephrology.* 11:2000.
- Lingwood C, Law H, Richardson S, Petric M, Brunton J, et al. Glycolipid Binding of Purified and Recombinant Escherichia coli Produced Verotoxin in Vitro. *Journal of Biological Chemistry.* 1987; 262(18):8834-8839.
- Lockman LA, Hunninghake DB, Krivit W, Desnick RJ. Relief of pain of Fabry's disease by diphenylhydantoin. *Neurology.* 1973;23(8):871-875.
- Malouf N, Kirkman HN, Buchanan PD. Ultrastructural Changes in Antenatal Fabry's Disease. *Scientific Proceedings.* 1976;13a.
- Management of Cancer Symptoms: Pain, Depression, and Fatigue. Summary, Evidence Report/Technology Assessment: Number 61, July 2002. Agency for Healthcare Research and Quality, Rockville, MD. <http://www.ahrq.gov/clinic/epcsums/csypsum.html>.
- Marguery MC, Giordano F, Parant M, Samalens G, Levade T, et al. Fabry's Disease: Heterozygous Form of Different Expression in Two Monozygous Twin Sisters. *Dermatology.* 1993; 187:9-13.
- Mayes JS, Cray EL, Dell VA, Scheerer JB, Sifers RN. Endocytosis of lysosomal alpha-galactosidase A by cultured fibroblasts from patients with Fabry disease. *American Journal of Human Genetics.* 1982; 34:602-10.
- Meroni M, Sessa A, Battini G, Tazzari S, Torri Tarelli L. Kidney involvement in Anderson-Fabry disease. *Contributions to Nephrology.* 1997;122:178-184.
- Mitsias P, Levine SR. Cerebrovascular complications of Fabry's disease. *Annals of Neurology.* 1996;40(1):8-17.
- Moore RA, Gavaghan D, Tramer MR, Collins SL, McQuay HJ. Size is everything—large amounts of information are needed to overcome random effects in estimating direction and magnitude of treatment effects. *Pain.* 1998;78:209-216.
- Nakamura T, Kaneko H, Nishino I. Angiokeratoma corporis diffusum (Fabry disease): Ultrastructural studies of the skin. *Acta Dermato-Venereologica.* 1981;61:37-41.
- Ohshima T, Schiffmann R, Murray GJ, Kopp J, Quirk JM, et al. Aging accentuates and bone marrow transplantation ameliorates metabolic defects in Fabry disease mice. *Proceeding of the National Academy of Sciences of the United States of America.* 1999;96(11):6423- 6427.

- Olson JL, Hostetter TH, Rennke HG, Brenner BM, Venkatachalam MA. Altered glomerular permselectivity and progressive sclerosis following extreme ablation of renal mass. *Kidney International*. 1982; 22:112-126.
- Oshima T, Asano K, Shibata S, Suzuki Y, Masuzawa M. Urinary neutral glycosphingolipid analysis of patients with Fabry's disease; rapid isocratic elution from high-performance liquid chromatography as per-o-benzoyl derivatives. *Biochemical and Biophysical Acta*. 1990;1043:157-160.
- Peters FP, Sommer A, Vermeulen A, Cheriex EC, Kho TL. Fabry's disease: a multidisciplinary disorder. *Postgraduate Medical Journal*. 1997;73(865):710-712.
- Rodriguez FH, Hoffman EO, Ordinario AT, Balige M. Fabry's Disease in a Heterozygous Woman. *Archives of Pathology and Laboratory Medicine*. 1985; 109:89-91.
- Rubin, D. *Multiple Imputation for non-response in surveys*. Wiley, New York, 1987.
- Rubin, D., *Using Propensity Scores to Help Design Observational Studies: Application to the Tobacco Litigation*. *Health Services & Outcomes Research Methodology* 2001; 2:169-188.
- Sakuraba H, Igarashi T, Shibata T, Suzuki Y. Effect of vitamin E and ticlopidine on platelet aggregation in Fabry's disease. *Clinical Genetics*. 1987; 31:349-54.
- Schatzki PF, Kipreas B, Payne J. Fabry's disease. Primary diagnosis by electron microscopy. *American Journal of Surgical Pathology*. 1979; 3:211-9.
- Shelley ED, Shelly WB, Kurczynski TW. Painful Fingers, Heat Intolerance, and Telangiectases of the Ear: Easily Ignored Childhood Signs of Fabry Disease. *Pediatric Dermatology*. 1995;12(3):215-219.
- Temple R, Ellenberg SS. Placebo-Controlled Trials and Active-Control Trials in the Evaluation of New Treatments. *Annals of Internal Medicine*. 2000; 133(6):455-463.
- Thurberg BL, Rennke R, Colvin RB, Dikman S, Gordon RE, Collins AB, Desnick RJ, O'Callaghan, M. Globotriaosylceramide accumulation in the Fabry kidney is cleared from multiple cell types after enzyme replacement therapy. *Kidney Int*. 2002; 62:1933-1946
- Tsakiris D. Rare Diseases in renal replacement therapy in the ERA-EDTA Registry. *Nephrology, Dialysis, Transplantation*. 1996;Vol 11:4 – 20.
- Tsutsumi O, Sato M, Sato Ko, Stao Ka, Mizumo M, Sakamoto S. Early Prenatal Diagnosis of Inborn Error of Metabolism: A Case Report of a Fetus Affected with Fabry's Disease. *Asia-Oceania Journal of Obstetrics and Gynaecology*. 1985;11(1):39-45.

Ullman MD, Pyeritz RE, Moser HW, Wenger DA, Kolodny EH. Application of "High-Performance" Liquid Chromatography to the Study of Sphingolipidoses. *Clinical Chemistry*. 1980;26(10):1499-1503.

U.S. Census Bureau, Census 2000. (<http://www.census.gov/prod/2002pubs/c2kprof00-us.pdf>)

von Scheidt W, Eng CM, Fitzmaurice TF, Erdmann E, Hubner G, et al. An Atypical Variant of Fabry's Disease with Manifestations Confined to the Myocardium. *New England Journal of Medicine*. 1991;324 (6):395-399.

Wuthrich RP, Weinreich T, Binswanger U, Gloor HJ, Candinas D, Hailemariam S. Should living related kidney transplantation be considered for patients with renal failure due to Fabry's disease? *Nephrology, Dialysis, Transplantation*. 1998;13:2934-2936.

Yanagawa Y, Sakuraba H. Cardiovascular manifestations in Fabry's disease – age-related changes in hemizygotes and heterozygotes. *Acta Paediatric Japan*. 1988;30(1):38-48.

Yoshitama T, Nakao S, Takenaka T, Teraguchi H, Sasaki T, et al. Molecular Genetic, Biochemical, and Clinical Studies in Three Families With Cardiac Fabry's Disease. *American Journal of Cardiology*. 2001; 87:71-75.

Zeidner KM, Desnick RJ, Ioannou YA. Quantitative Determination of Globotriaosylceramide by Immunodetection of Glycolipid-Bound Recombinant Verotoxin B Subunit. *Analytical Biochemistry*. 1999; 267:104-113.