Long-Term Safety and Efficacy of Enzyme Replacement Therapy for Fabry Disease

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Elsewhere, we reported the safety and efficacy results of a multicenter phase 3 trial of recombinant human α-galactosidase A (rh-αGalA) replacement in patients with Fabry disease. All 58 patients who were enrolled in the 20-wk phase 3 double-blind, randomized, and placebo-controlled study received subsequently 1 mg/kg of rh-αGalA (agalsidase beta, Fabrazyme, Genzyme Corporation) biweekly in an ongoing open-label extension study. Evidence of long-term efficacy, even in patients who developed IgG antibodies against rh-αGalA, included the continuously normal mean plasma globotriaosylceramide (GL-3) levels during 30 mo of the extension study and the sustained capillary endothelial GL-3 clearance in 98% (39/40) of patients who had a skin biopsy taken after treatment for 30 mo (original placebo group) or 36 mo (original enzyme-treated group). The mean serum creatinine level and estimated glomerular filtration rate also remained stable after 30–36 mo of treatment. Infusion-associated reactions decreased over time, as did anti-rh-αGalA IgG antibody titers. Among seroconverted patients, after 30–36 mo of treatment, seven patients tolerized (no detectable IgG antibody), and 59% had ⩾4-fold reductions in antibody titers. As of 30 mo into the extension trial, three patients were withdrawn from the study because of positive serum IgE or skin tests; however, all have been rechallenged successfully at the time of this report. Thus, enzyme replacement therapy for 30–36 mo with agalsidase beta resulted in continuously decreased plasma GL-3 levels, sustained endothelial GL-3 clearance, stable kidney function, and a favorable safety profile.

Introduction

Fabry disease (MIM 301500) is an X-linked lysosomal storage disease resulting from the deficient activity of α-galactosidase A and from the progressive accumulation of globotriaosylceramide (GL-3) and related glycosphingolipids in the plasma and in tissue lysosomes throughout the body (Desnick et al. 2001). In classically affected males, vascular endothelial GL-3 accumulation in the kidney, brain, and heart leads to early demise due to renal failure, stroke, and cardiovascular disease (Colombi et al. 1967; Desnick et al. 2001; MacDermot et al. 2001).

A phase 1/2 clinical trial demonstrated that five biweekly doses of 1.0 mg/kg of recombinant human α-galactosidase A (rh-αGalA) (agalsidase beta, Fabrazyme, Genzyme Corporation) reduced the accumulated GL-3 from the vascular endothelium of the kidney,

heart, and skin of classically affected males (Eng et al. 2001*a*). Subsequently, a pivotal phase 3 double-blind, randomized, placebo-controlled, and multicenter trial evaluated the safety and efficacy of rh-αGalA in 58 classically affected patients, including 2 females (Eng et al. 2001*b*). At the end of the 20-wk double-blind study, 69% (20/29) of the agalsidase beta–treated patients showed GL-3 clearance to normal or near normal levels in renal capillary endothelial cells, whereas none (0/29) of the placebo-treated patients had cleared the glycosphingolipid (*P* < .001). All 58 patients enrolled subsequently in an ongoing extension trial, and, after an additional 6 mo of treatment, 100% (24/24) of the patients previously treated with placebo and 92% (23/25) of the patients previously treated with agalsidase beta had cleared GL-3 from their renal endothelial cells (Eng et al. 2001*b*). Here, we report on the continued safety and efficacy of agalsidase beta replacement in patients, after 30 mo of an ongoing open-label extension trial.

Subjects and Methods

Patients

All patients who completed successfully the phase 3 double-blind trial were eligible to enroll in the extension

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study and were required to provide written informed consent prior to inclusion in the study (see Eng et al. [2001*b*] for phase 3 inclusion/exclusion criteria and patient demographics). The open-label extension study was approved by the institutional review boards and/or ethics committees of all participating centers.

Study Protocol

In the open-label extension study, all patients received 1 mg/kg (0.9–1.1 mg/kg) of agalsidase beta every 2 wk. Prior to infusion, patients received 500–1,000 mg of acetaminophen. Also, some patients were pretreated with an antihistamine approved by the investigator. A few patients received ibuprofen, prednisone, or both, as pretreatment to minimize infusion-associated reactions (IARs). Infusion rates were increased as tolerated, resulting in a significant reduction in infusion duration, which, in some patients, decreased to 90 min. Patients were eligible to transfer to local sites, after the first 4 mo in the extension study. In addition, home infusions were offered to patients who were clinically stable with no IARs within the last 4 mo (eight infusions), who were at the same infusion rate for a minimum of four infusions, and who had no ongoing serious adverse events.

Serum IgE tests and, if needed, skin tests were performed on patients who developed IARs suggestive of an IgE-mediated response. If a preinfusion serum sample was not available, a serum sample was drawn 3–8 d after the reaction, for serum IgE testing. Skin testing involved scarification (prick) tests and, if negative, intradermal testing. Patients were withdrawn from the study by protocol if their serum IgE or skin test was positive and were eligible for a cautious rechallenge with agalsidase beta, with the use of the following infusion scheme: the first two infusions of agalsidase beta were administered at a dose of 0.5 mg/wk at a starting rate not exceeding 0.01 mg/min, which then could be doubled every 30 min for the remainder of the infusion (to a maximum rate of 0.25 mg/kg/min) if no significant or intolerable infusion-associated symptoms occurred. Subsequently, the dose could be increased to 1.0 mg/kg of agalsidase beta intravenously every other week for the duration of the study. Pretreatment was not permitted for the first four infusions in this protocol, to allow early recognition of acute systemic reactions. Antihistamines, antipyretics, β-agonists, or steroids were permitted for subsequent infusions, in consultation with Genzyme Pharmacovigilance. In addition, patients on beta blockers were asked to stop this medication 2 d prior to the infusion until 12 h after the infusion.

Results reported here document the first 30 mo of agalsidase beta treatment in the extension study. Patients who received agalsidase beta in the double-blind study have received 36 mo of treatment and are designated as the "agalsidase beta/agalsidase beta" group. Patients who received placebo in the double-blind study have received 30 mo of treatment and are designated as the "placebo/agalsidase beta" group.

Assessment of Skin Biopsies

Skin biopsies were obtained by punch biopsy (3 mm) at the baseline of the placebo-controlled trial; at completion of the placebo-controlled trial (20 wk); at 6 mo, 12 mo, and 18 mo into the extension trial; and annually thereafter. Tissue sections (1 μm) were stained with methylene blue-azure II, as described elsewhere (Eng et al. 2001*b*). Specimens were observed via light microscopy for accumulation of GL-3. Specimens with no microvascular endothelial deposits of GL-3 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. Specimens that had large accumulations of inclusions, with some clusters at the juxtanuclear region and around cytoplasmic borders, and bulging of the vessel lumens were given a score of 3.

Clinical and Biochemical Assessments

The following assessments were performed every 6 mo during the extension study: (1) vital signs, physical examination, routine blood chemistries, hematology, and urinalysis; (2) serum creatinine and the mean estimated glomerular filtration rate (GFR), calculated by the Modification of Diet in Renal Disease Study (MDRD) equation (Levey et al. 2000) on the basis of patients' age, race, sex, and serum-creatinine values; (3) proteinuria (determined with the use of the urine protein:urine creatinine ratio, which represents the approximate urine protein excretion in g/d); (4) 12-lead EKG; (5) health status, evaluated with the Short Form-36 (SF-36) Health Status Survey (Ware et al. 1997); and (6) pain score, evaluated with the short form McGill pain questionnaire (Melzack 1987). Echocardiography was performed annually.

Plasma GL-3 concentrations were determined initially in the phase 3 trial with the use of an ELISA method (Zeidner et al. 1999) at the following times: baseline, 14 wk, and 20 wk of the phase 3 double-blind study and entry, 6 mo, and 12 mo of the open-label extension study (Eng et al. 2001*b*). Samples obtained at 18 mo, 24 mo, and 30 mo into the extension study, along with archived baseline samples of the double-blind study, were analyzed with the use of a more sensitive mass-spectrometric assay (Genzyme Data on File). In brief, total lipids were extracted from plasma via chloroform/methanol liquid-liquid extraction. Neutral glycosphingolipids were isolated from the organic phase layer and further purified by C_{18} chromatography. The purified

extract was dried and reconstituted in methanol, and the total GL-3 was quantified by analyzing the sample with a multiple reactions monitoring mode LC/MS/MS (liquid chromatography/tandem mass spectroscopy) . The constant neutral loss of the galactosyl group (*m/z* 162) from GL-3 during MS/MS analysis has permitted both confirmation of identity and development of a sensitive quantification scheme. Total GL-3 is quantified by the sum of the 10 major isoforms (C16:0-, C18:0-, C20:0-, C22:0-, C22:1-, C22:0-OH, C24:0-, C24:1-, C24:0-OH, and C26:0-GL-3) measured in the plasma sample with the use of C17:0, a nonnaturally occuring isoform of GL-3, as an internal standard. The upper limit of normal for plasma GL-3, with the use of the more sensitive mass-spectrometric assay, was 7.03 μg/ml, on the basis of the estimated 99th percentile value from 205 normal plasma samples from a blood bank (the mean $\pm$ SD of the 205 samples was 3.5 $\pm$ 1.3 μg/ml).

Antibodies

Blood was drawn prior to every other infusion, and the serum was screened for the presence of IgG antibodies against rh-αGalA with the use of an ELISA specific for rh-αGalA. The results were confirmed by a radioimmunoprecipitation (RIP) assay. Quantitation of the antibody was done by titrating the antibody reactivity with the ELISA assay, following a 2-fold dilution scheme starting at 1/100. Normal distribution studies of >100 normal human sera have shown that this initial dilution (1/100) had minimal reactivity in the ELISA and was essentially "background" for normal serum. If a patient did not seroconvert throughout the entire study period, then the patient was defined as having no immune response ("seronegative"). If a patient seroconverted and later ceased producing IgG antibodies, as determined by the ELISA assay within the normal range and by two consecutive negative confirmatory RIP assays, then the patient was defined as having "tolerized." The remaining patients who seroconverted without tolerizing were classified as follows. "Low responders" were defined as those who did not tolerize and whose highest titer value was ≤800 (i.e., 1:8 above background). Patients who did not tolerize and had at least one titer value >800 and a 4-fold decrease in titer from the peak to the last value were designated as having a "downward trend." Patients for whom at least one titer was >800 and the highest titer to date was achieved at the last visit were given the titer classification of "highest titer to date." Patients who seroconverted but did not fit any of the above criteria were defined as having "plateaued."

Statistical Analysis

For skin biopsies, the exact binomial matched pairs procedure was used to determine if there was a statistically significant difference in the proportion of patients showing a change from entry to 30 mo of treatment in the extension study. Two-tailed tests were used for all analyses. One-sample *t*-tests were used to compare the mean changes in glomerular filtration, pain, and SF-36 scores within groups, from entry to 30 mo into the extension study. $P \leq .05$ was considered statistically significant.

Results

Patient Characteristics

All 58 patients who participated in the phase 3 double-blind, randomized, and placebo-controlled trial enrolled in the open-label extension study. Their baseline characteristics were described elsewhere (Eng et al. 2001*b*). By month 30 of the open-label extension trial, eight patients had withdrawn (because of voluntary withdrawal [four patients], protocol-specified criteria [three], or death [one]). Three patients, who were withdrawn by protocol, had a positive serum IgE or skin test. At the time of this report, all three patients were rechallenged with agalsidase beta, either with the commercial drug or in a rechallenge protocol, and continue to be treated (the EMEA approved Fabrazyme in June 2001, and the FDA approved Fabrazyme in April 2003). No patient experienced anaphylaxis. One 43-year-old patient died 10 d after infusion 29; he collapsed at home, and resuscitation was unsuccessful. He had a history of a myocardial infarction at age 30 years and a pacemaker for severe bradycardia at age 43 years, and he had left-ventricular hypertrophy and heart failure. An autopsy revealed acute heart failure and severe cardiovascular involvement consistent with Fabry disease.

Efficacy of Long-Term Therapy

As shown in figure 1, the mean values for plasma GL-3 were in the normal range at 18 mo and have remained normal after 30–36 mo of treatment for the original placebo and the agalsidase beta–treated groups in the double-blind study, respectively. The mean plasma GL-3 concentrations, determined by the mass spectrometric assay, were 4.9 μg/ml and 5.0 μg/ml for the placebo/ agalsidase beta and agalsidase beta/agalsidase beta groups after 30–36 mo of treatment, respectively. The mean plasma GL-3 levels were reduced into the normal range in 94% (44/47) of the patients who had plasma data available after 30–36 mo of treatment (fig. 1). The three outliers had values of 9.1, 8.2, and 8.0 μg at 30 mo, compared with baseline values of 15.6, 16.6, and 14.0 μg, respectively. None of the patients with the elevated plasma GL-3 levels at month 30 had a missed infusion prior to the plasma GL-3 sampling. Two of the three patients with elevated plasma GL-3 levels at month 30 had normalized their plasma GL-3 levels at a sub-
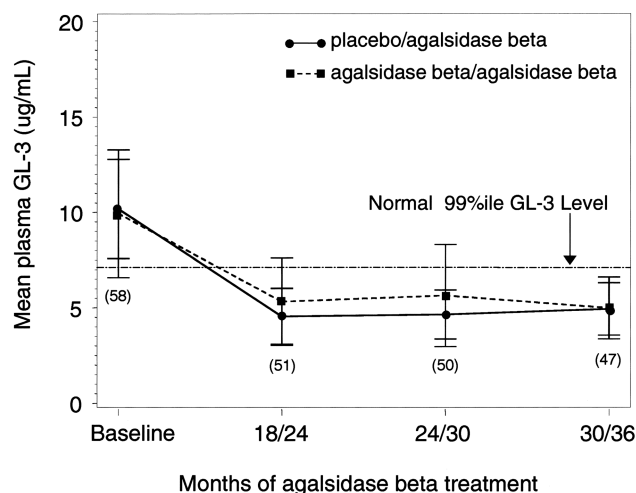
Figure 1   Mean (±SD) plasma GL-3 concentration (μg/ml), measured by mass spectrometry at baseline and at 18 mo, 24 mo, and 30 mo into the phase 3 extension study, showing normalization of mean plasma GL-3 levels after agalsidase beta therapy. At 30 mo into the extension study, 94% of patients had normal plasma GL-3 values. The mean (±SD) values of the control group were 3.5 (±1.3) μg/ml. The number of patients is indicated in parentheses below the graphed points.

sequent time; the third patient did not have a plasma GL-3 level available at the time of this report.

Skin biopsies were obtained from 40 patients after 30 mo of the extension study. Of these, 98% (39/40) had capillary endothelial cell scores of 0, consistent with continuous GL-3 clearance (fig. 2). The single patient with a nonzero score at 30 mo of treatment had a score of 1 (his baseline score was 2), whereas his serum creatinine was 1.0 mg/dl, his estimated GFR was 94.9 ml/min/1.73 m², his plasma GL-3 level was 5.8 μg/ml, and his antibody titers decreased over time (i.e., a downward trend).

Renal function, assessed by mean serum creatinine and estimated GFR, remained stable throughout the 30-mo extension study for both study groups (fig. 3A). At entry to the extension trial, the mean serum creatinine concentrations for both the placebo/agalsidase beta and agalsidase beta/agalsidase beta groups were normal (0.8 and 0.9 mg/dl, respectively) and remained normal through 30 mo of the extension study (mean serum creatinine of 0.9 and 1.0 mg/dl, respectively). Of note, after 30 mo of treatment with Fabrazyme in the phase 3 open-label extension study, three patients had a >50% increase in their serum creatinine levels, with the last value being >1.4 mg/dl from the start of treatment. The increase in SD over time is reflective of the three patients who had a >50% increase in their serum creatinine value from the start of treatment. These are the only three patients in the study who, at baseline, had both prominent glomerulosclerosis and a urinary protein:urinary creatinine

ratio >2.0 (representing an approximate urine protein excretion of >2 g/d). In addition, all three patients were age >40 years, the age range at which most patients with Fabry disease demonstrate a decline in renal function (Colombi et al. 1967; Branton et al. 2002; Thadhani et al. 2002). When these three patients were removed from the serum creatinine calculation, the mean values for the placebo/agalsidase beta and agalsidase beta/agalsidase beta groups were 0.8 and 0.9 mg/dl, respectively (fig. 3B).

An assessment of the number of glomeruli showing focal segmental glomerulosclerosis, the number showing global glomerulosclerosis, and the number that appeared normal had been performed on the 48 patients who had renal biopsies at all three earlier-specified times (baseline of the double-blind study, end of the double-blind study, and 6 mo into the open-label extension study). Of these 48 patients, 43 had glomeruli present in both the baseline and 6-mo extension biopsies. Review of these analyses demonstrated that, for 14% (6/43) of patients, >50% of all observed glomeruli demonstrated either focal segmental glomerulosclerosis or global glomerulosclerosis in the baseline kidney biopsy (table 1). The three patients, discussed above, who had deterioration of renal function are in this group of six patients.

At entry into the extension trial, the mean estimated GFRs for both the placebo/agalsidase beta and agalsidase beta/agalsidase beta groups (138.3 and 109.6 ml/min/1.73 m², respectively) were normal and remained normal through 30 mo of the extension study (mean estimated GFRs of 129.5 and 107.1 ml/min/1.73 m², respectively). Of note, a subset of 10 patients in this trial had low estimated GFR values (<90 ml/min/1.73 m²) prior to the start of treatment with agalsidase beta. For these 10 patients, the mean (±SD) GFR values prior to treatment and 30–36 mo later were 68.6 (±19.65) and 79.6 (±6.27) ml/min/1.73 m², respectively. After 30–36 mo of agalsidase beta treatment, 7 (70%) of the 10 patients had either stabilized their estimated GFR values (GFR ± 20%) or improved.

The median urinary protein-to-creatinine ratio, for both treatment groups combined, was 0.221 at baseline of the double-blind study and remained stable at 0.198 after 30–36 mo of rh-α-GalA therapy (*N* = 27; all patients who had data available at both times), indicating a stabilization in urine protein excretion. As shown in figure 4, the few patients who had an increase in urine protein excretion had relatively high proteinuria at baseline, suggesting that baseline factors may play a role in response to therapy.

A slight improvement was observed during the open-label extension study in both treatment groups for most SF-36 parameters; however, these changes were not statistically significant. Pain scores, as assessed by the McGill pain questionnaire, remained low throughout the
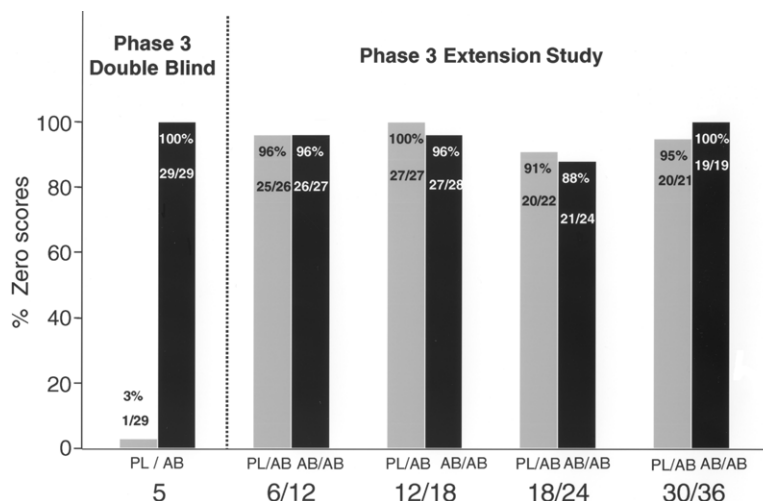
Figure 2 GL-3 clearance in dermal superficial endothelial cells in phase 3 double-blind trial and extension study (% zero scores). Clearance of GL-3 from the dermal capillaries was sustained after prolonged agalsidase beta therapy. PL = placebo; AB = agalsidase beta.

extension study. It should be noted that patients had low pain scores at entry (the mean total pain scores were 2.0 for the placebo/agalsidase beta group and 4.1 for the agalsidase beta/agalsidase beta group; the highest possible pain score is 45).

Starting at month 24 of the extension study, the investigators asked their patients to report pain-medication usage in the preceding 3 mo. Four patients in the agalsidase beta/agalsidase beta group and one patient in the placebo/agalsidase beta group stopped all pain medications between months 27 and 30. Four patients in the agalsidase beta/agalsidase beta group and two patients in the placebo/agalsidase beta group had reduced the dose and/or the frequency of their pain medications. However, most patients taking pain medications ($n =$ 34) used them on an as-needed basis.

Safety of Long-Term Therapy

The most common related adverse events occurred during the infusions and consisted of rigors, temperature-change sensation (feeling cold or warm), fever, nausea, headache, and nasal congestion. These adverse events were generally mild and tolerable, and patients were managed with infusion rate reduction and, if needed, with additional medications. Initially, IARs occurred usually between infusions 5 and 7 (coinciding with seroconversion). Only one of the seven seronegative patients experienced an IAR. As shown in figure 5, the percentage of patients experiencing IARs decreased over time. Eight patients experienced serious adverse events, which were most often infusion-associated events, including tachycardia, hypertension, urticaria, chest pain and/or throat tightness, fevers, and rigors.

It should be noted that the current phase 3 extension study was not designed to assess the effect of enzyme replacement therapy (ERT) on clinical outcomes. Data on stroke and cardiovascular events were not collected as part of the study but rather as adverse events. Hence, detailed information was not available. Nonetheless, 8.6% (5/58) of patients were reported to have cerebrovascular events (stroke or transient ischemic attack) at the time of this report. One of these five patients had an extensive past history of stroke. The other four patients, presumably, did not have a prior history of stroke. These patients did not have renal failure or cardiac complications during the study. The lack of a placebo group makes interpretation of these data difficult. Review of a large historical database of patients with Fabry aged ≥16 years shows that 13.7% (57/415) of patients had a cerebrovascular event (Genzyme Data on File).

The rate of seroconversion among enzyme-treated patients was 89.7% (52/58), and, among those who seroconverted, the median time to seroconversion was ~6 wk, with a median time of 63 d to peak titer. After 30 mo of the extension study, 10.3% (6/58) of patients remained seronegative. There were no obvious characteristics that might help to identify prospectively patients who will not seroconvert. Of the 52 patients who seroconverted, 1 patient was excluded from the longitudinal analysis because he withdrew from the phase 3 open-label study after infusion 8, and comparative antibody titers were available only for a short duration of time. Of the remaining 51 patients, 13.7% (7/51) tolerized, 3.9% (2/51) were low responders, 19.9% (10/51) plateaued, 3.9% (2/51) had their highest titers at their last visit, and 58.8% (30/51) had downward trends in their antibody titers. For the two patients whose highest titers
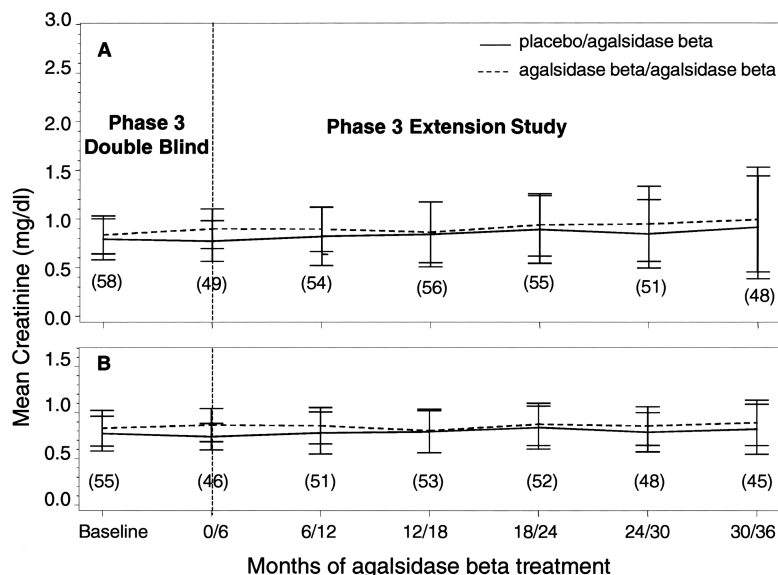
Figure 3 Mean (±SD) serum creatinine concentrations (mg/dl) at baseline during the phase 3 extension. *A*, Mean serum creatinine concentration, normal at baseline and during the phase 3 double-blind trial and phase 3 extension study. The increase in SD over time reflects the three patients who had >50% increase in serum creatinine from the start of treatment with agalsidase beta. *B*, Mean (±SD) serum creatinine concentration after the three patients with advance disease were removed from the analysis. The number of patients is indicated in parentheses below the graphed points.

were at the last visit, there has been no decline in efficacy of treatment for either subject, as evidenced by their skin biopsy histologic scores of 0 and their normal plasma GL-3 (6.2 and 6.2 μg/ml, respectively) and serum creatinine (0.8 and 1.2 mg/dl, respectively) levels at month 30. Of note, one of the two females in the study was a low responder (peak titer = 400) and tolerized, whereas the other remained seronegative throughout the study.

After the double-blind study, progressive increases in the rate of administration were allowed at all sites. At month 30 of the extension study, 93% (54/58) of patients completed one or more infusions in ≤2.5 h, with a mean infusion time of 2.2 h, and 78% (45/58) of patients completed one or more infusions in ≤2.0 h.

Discussion

In Fabry disease, affected males with the classic phenotype uniformly develop renal disease, typically by 35–45 years of age, with an average of ~42 years (Colombi et al. 1967; Desnick et al. 2001; Branton et al. 2002; Thadhani et al. 2002). Since early renal failure primarily results from GL-3 accumulation in endothelial and glomerular cells, therapeutic endeavors have focused on stabilizing or reversing the renal pathology (Schiffmann et al. 2000; Eng et al. 2001*a*). A phase 3 double-blind, randomized, and placebo-controlled trial and an open-label extension study described the safety and effectiveness of rh-αGalA replacement therapy using agalsidase

beta (Fabrazyme, Genzyme Corporation) at 1 mg/kg biweekly (Eng et al. 2001*b*). The primary endpoint—the clearance of accumulated GL-3 to normal or near normal levels from renal microvascular endothelial cells—was documented in 96% (47/49) of patients after 6–12 mo of agalsidase beta treatment (Eng et al. 2001*b*; Thurberg et al. 2002). In addition, accumulated GL-3 deposits were reduced to normal or near normal levels in renal mesangial cells, glomerular capillary endothelium, noncapillary endothelium, and interstitial cells. GL-3 deposits were reduced also in vascular smooth muscle cells, tubular epithelium, and podocytes, although at a slower rate (Thurberg et al. 2002). In addition, the plasma GL-3 levels normalized and the mean serum creatinine and estimated GFR remained stable after 6–12 mo of agalsidase beta treatment (Eng et al. 2001*b*; Thurberg et al. 2002). Here, we report the 30-mo results of the phase 3 open-label extension study, which demonstrate the continued safety and efficacy of agalsidase beta replacement therapy at 1 mg/kg biweekly.

A major question addressed by the extension study was whether long-term ERT could sustain the dramatic GL-3 clearance described in the double-blind study and in the first 6 mo of the extension study (Eng et al. 2001*b*) and, in particular, whether the IgG antibodies against the recombinant enzyme would impair efficacy. In addition, since renal failure is the most uniform and devastating feature in classic Fabry disease, the long-term effects on kidney function in these patients, some of

Table 1

Clinical Characteristics of Patients with Sclerosis of ≥50% Glomeruli at Baseline

Patient	Treatment Group	Baseline Age (years)	Ratio of Baseline Urine Protein to Urine Creatinine[a]	Serum Creatinine[b,c] (mg/dl)	30-Mo Serum Creatinine (mg/dl)	% Increase in Serum Creatinine[c,d]
110	Placebo/agalsidase beta	26	NA	1.0	1.4	40
119	Placebo/agalsidase beta	48	3.56	1.6	3.1	93.8
707	Agalsidase beta/agalsidase beta	45	2.92	.7	1.8[e]	157.1
708	Placebo/agalsidase beta	41	1.48	.8	.7	−12.5
709	Placebo/agalsidase beta	30	.79	1.1	1.2	9.1
804	Agalsidase beta/agalsidase beta	42	2.03	1.1	3.2	190.9

[a] NA = not available.

[b] Prior to the start of treatment with agalsidase beta.

[c] The start of treatment for the agalsidase beta/agalsidase beta patients is the baseline of the phase 3 placebo-controlled study, and the start of treatment for the placebo/agalsidase beta patients is the entry of the phase 3 extension study.

[d] From the start of treatment with agalsidase beta to 30 mo into the extension study.

[e] 24-mo value (30-mo value not available).

whom initially had histologic evidence of severe renal damage and significant proteinuria, were evaluated.

Biochemical evidence for the persistent effectiveness of agalsidase beta therapy at 1 mg/kg biweekly was the continuous normalization of the mean plasma GL-3 levels in the patient groups who had 30–36 mo of treatment (fig. 1). In the phase 3 double-blind study and in the first 6 mo of the extension study, the mean plasma levels were reduced to nondetectable levels with the use of an ELISA method to quantitate plasma GL-3. A new, more sensitive and robust mass-spectrometric method was employed to reassay the original baseline specimens and those obtained during the course of the extension study. The mean plasma GL-3 levels were reduced continuously into the normal range in 94% (44/47) of the patients who had plasma data available after 30–36 mo of treatment (fig. 1). In addition, histologic evidence for persistent GL-3 clearance from vascular endothelial cells was provided by examination of serial skin biopsies. In 98% of the 40 patients who had skin biopsies after 30–36 mo of treatment, GL-3 clearance was achieved and maintained (fig. 2). Only one patient had a score of 1, and his baseline score was 2.

The average age of the patients at baseline of the phase 3 double-blind study was 30.2 years, and, as expected, their mean serum creatinine was normal: 0.8 ± 0.2 mg/dl. After 30–36 mo of treatment, the mean serum creatinine levels were 0.9 and 1.0 mg/dl for the placebo/agalsidase beta and agalsidase beta/agalsidase beta groups, respectively (fig. 3A). Nonetheless, three patients, all aged >40 years and with significant baseline pathology, as assessed by the presence of glomerular sclerosis and nephrotic-range proteinuria, had progression of renal insufficiency. Indeed, when these three patients with advance diseased were excluded from the serum creatinine analysis, the mean and SD decreased (fig. 3B), indicating continued stable renal function in

the remaining patients. These data suggest that treatment before extensive renal damage occurs is critical and that there may be a "point of no return," in which impaired glomeruli will progress irreversibly to failure. Clearly, prevention of renal disease requires early therapeutic intervention in all classically affected males and substantially affected carriers (Desnick et al. 2003). To wait for elevated serum creatinine levels, when more than half the renal function has been lost, before initiating treatment may be counterproductive.

On the basis of our data, we cannot determine the effect of treatment on cerebrovascular risk. Given that there is likely to be at least some irreversible pathologic damage in patients first treated in adulthood, such patients are probably at increased cardiac and cerebrovascular risk. Therefore, every effort should be made to aggressively control other risk factors, such as hyperlipidemia, hypertension, and smoking. Prophylactic aspirin is prescribed commonly for men >35 years old with Fabry; however, the effectiveness of this therapy in this population is currently unknown.

Improvement in pain is notoriously difficult to assess. Indeed, in the double-blind placebo-controlled trial, statistically significant improvement in pain scores was observed in both placebo- and agalsidase-treated groups. Nonetheless, during the phase 3 extension trial, some patients reported a decrease in or cessation of the use of pain medications, suggesting that longer treatment decreased the frequency and/or intensity of their pain and/or the need for pain-relieving medications. In fact, most patients required pain medications only on an as-needed basis.

Generally, infusions of agalsidase beta were well-tolerated by patients, with the frequency of IARs declining over the course of the study. There were no reports of anaphylaxis, and the three patients who were withdrawn by protocol, because of a positive serum IgE
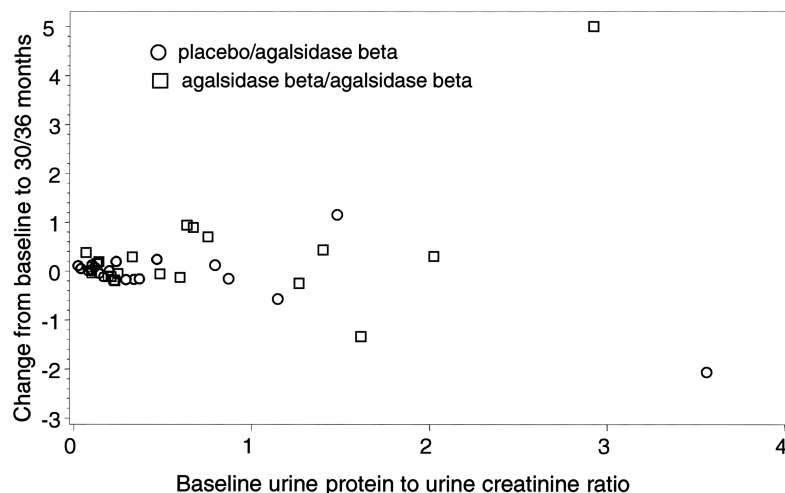
Figure 4 Urinary protein excretion. Urine protein:urine creatinine, the ratio representing the approximate urine protein excretion in g/d, remained stable from the baseline of the double-blind study to 30 mo into the extension study. Change in urine protein excretion is associated with baseline proteinuria. This figure presents data on all 27 patients for whom data were available at both times.

or skin test, have been rechallenged subsequently with success. Thus, the clinical significance of these tests remains unclear.

IgG antibodies were found in ~90% of treated patients, and the median time to serocoversion was ~6 wk. This was expected, since most classically affected males with Fabry disease are negative for cross-reacting immunologic material (CRIM), as a result of frameshift, missplicing, stop codon, or unstable missense mutations. However, 13.5% of the seroconverted patients tolerized (no detectable IgG antibodies), and the majority of seroconverted patients demonstrated a ⩾4-fold reduction in antibody titer over the 30-mo extension study. Notably, seroconversion did not affect long-term efficacy of agalsidase beta treatment, as evidenced by persistently normalized plasma GL-3 levels, sustained GL-3 clearance in skin capillaries, and stable renal function. In comparison, ~15% of patients with type 1 Gaucher disease, who have residual acid β-glucosidase activity and are CRIM-positive, raise nonneutralizing IgG antibodies during ERT (Richards et al. 1993; Rosenberg et al. 1999). However, experience with ERT in type 1 Gaucher disease for >10 years has demonstrated that seroconverted patients developed tolerance and that therapeutic efficacy was not impaired by the presence of IgG antibodies (Rosenberg et al. 1999).

Moreover, recent clinical experiences and non–placebo-controlled studies have indicated that ERT with agalsidase beta stabilized renal function (De Schoenmakere et al. 2003), improved cardiac function (Waldek 2003; Weidemann et al. 2003), and improved quality of life (Guffon and Fouilhoux, in press). Several investigators participating in this study have also noted,

among some patients, improvement in specific clinical manifestations, including fatigue, exercise tolerance, abdominal pain, diarrhea, ability to sweat, and lymphedema. In addition, most patients report decreased and/or less-frequent Fabry pain, but these findings have varied among patients.

Clearly, additional experience is required to demonstrate the long-term effectiveness of agalsidase beta therapy on renal, cardiac, and cerebrovascular manifestations, which are the major causes of premature death in this disease. However, it has been recommended by physicians who are expert in Fabry disease that all affected males and carriers with substantial manifestations should be treated, with treatment being initiated as early as possible to prevent disease manifestations (Desnick et al. 2003). A Fabry disease registry has been established to follow patients long term and to assess the effect of ERT on this disease (Fabry Registry).

In summary, this open-label extension study of agalsidase beta replacement extends the findings of the double-blind, randomized, and placebo-controlled trial and demonstrates that, after 30–36 mo of treatment, ERT continued to be safe and effective. The continuous efficacy was evaluated on the basis of the relatively noninvasive serial determinations of substrate accumulation, including persistently normalized plasma GL-3 levels and the continuous clearance of GL-3 deposits from the vascular endothelium of biopsied skin. The absence of reaccumulated GL-3 in plasma and vascular endothelial cells indicates that the replaced enzyme, even in the presence of IgG antibodies, continuously cleared the plasma and the vascular endothelial cells that are the major target sites of pathology in Fabry disease (Desnick et al. 2001).
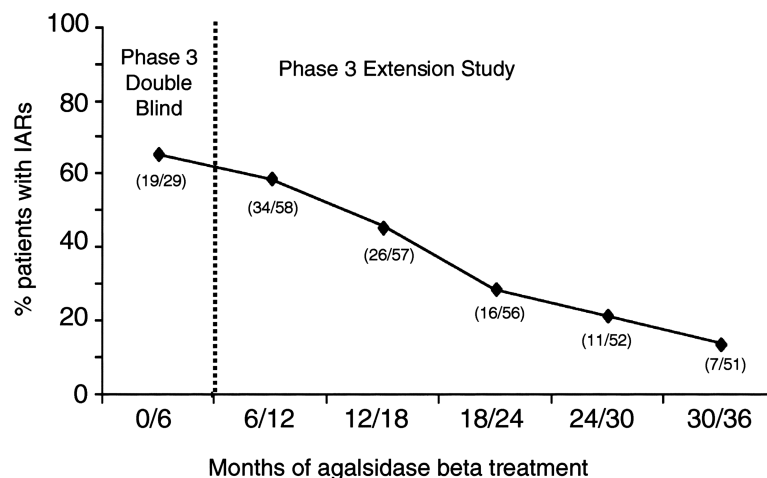
Figure 5   Percentage of patients experiencing IARs during the phase 3 double-blind and phase 3 extension studies, decreasing over time. The number of patients is indicated in parentheses below the graphed points.

Acknowledgments

Electronic-Database Information

URLs for data presented herein are as follows:

Fabry Registry, http://www.lsdregistry.net/fabryregistry
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omin/

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