

ORIGINAL ARTICLE

Treatment of Fabry's Disease with the Pharmacologic Chaperone Migalastat

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ABSTRACT

BACKGROUND

Fabry's disease, an X-linked disorder of lysosomal α -galactosidase deficiency, leads to substrate accumulation in multiple organs. Migalastat, an oral pharmacologic chaperone, stabilizes specific mutant forms of α -galactosidase, increasing enzyme trafficking to lysosomes.

METHODS

The initial assay of mutant α -galactosidase forms that we used to categorize 67 patients with Fabry's disease for randomization to 6 months of double-blind migalastat or placebo (stage 1), followed by open-label migalastat from 6 to 12 months (stage 2) plus an additional year, had certain limitations. Before unblinding, a new, validated assay showed that 50 of the 67 participants had mutant α -galactosidase forms suitable for targeting by migalastat. The primary end point was the percentage of patients who had a response ($\geq 50\%$ reduction in the number of globotriaosylceramide inclusions per kidney interstitial capillary) at 6 months. We assessed safety along with disease substrates and renal, cardiovascular, and patient-reported outcomes.

RESULTS

The primary end-point analysis, involving patients with mutant α -galactosidase forms that were suitable or not suitable for migalastat therapy, did not show a significant treatment effect: 13 of 32 patients (41%) who received migalastat and 9 of 32 patients (28%) who received placebo had a response at 6 months ($P=0.30$). Among patients with suitable mutant α -galactosidase who received migalastat for up to 24 months, the annualized changes from baseline in the estimated glomerular filtration rate (GFR) and measured GFR were -0.30 ± 0.66 and -1.51 ± 1.33 ml per minute per 1.73 m^2 of body-surface area, respectively. The left-ventricular-mass index decreased significantly from baseline (-7.7 g per square meter; 95% confidence interval [CI], -15.4 to -0.01), particularly when left ventricular hypertrophy was present (-18.6 g per square meter; 95% CI, -38.2 to 1.0). The severity of diarrhea, reflux, and indigestion decreased.

CONCLUSIONS

Among all randomly assigned patients (with mutant α -galactosidase forms that were suitable or not suitable for migalastat therapy), the percentage of patients who had a response at 6 months did not differ significantly between the migalastat group and the placebo group. (Funded by Amicus Therapeutics; ClinicalTrials.gov numbers, NCT00925301 [study AT1001-011] and NCT01458119 [study AT1001-041].)

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FABRY'S DISEASE IS A RARE, PROGRESSIVE, and devastating X-linked disorder caused by the functional deficiency of lysosomal α -galactosidase.¹ The resultant accumulation of glycosphingolipids, predominantly globotriaosylceramide (GL-3), can lead to multisystem disease and early death.²

Binding of the pharmacologic chaperone migalastat to the active site of α -galactosidase stabilizes certain mutant enzymes, thus facilitating proper trafficking to lysosomes, where dissociation of migalastat allows α -galactosidase to catabolize accumulated substrates.³⁻⁷ In patients with mutant enzymes that are identified with the validated assay, orally administered migalastat may be an alternative treatment option for addressing certain unmet medical needs associated with enzyme-replacement therapy — for example, antibody formation to enzyme-replacement therapy, which may exacerbate infusion-associated reactions and interfere with efficacy.⁸⁻¹⁰

As an orally administered small-molecule agent (see the Supplementary Appendix, available with the full text of this article at NEJM.org), migalastat may obviate the burden of lifelong administration of enzyme-replacement infusions every 2 weeks and immunogenicity associated with enzyme-replacement therapy. The higher volume of distribution of migalastat (76.5 to 133 liters of total body water in adults)¹¹ than of recombinant α -galactosidase^{12,13} hints at the possibility of better diffusion in organs and tissues.⁴ Theoretically, chaperoning misfolded α -galactosidase to lysosomes may better mimic natural enzyme trafficking and result in more consistent α -galactosidase activity than enzyme-replacement infusions every 2 weeks. We now report the results of a phase 3 study that evaluated the efficacy and safety of migalastat in male and female patients with Fabry's disease.

METHODS

STUDY DESIGN

After eligibility had been determined and baseline assessments performed during a 2-month screening period, patients were randomly assigned to stage 1, which included 6 months of double-blind administration of migalastat hydrochloride (150 mg) or placebo every other day. All patients who completed stage 1 were eligible to receive open-label migalastat in stage 2 (months 6 through

12) and for an additional year (months 12 through 24) thereafter (study AT1001-011) (Fig. S1 in the Supplementary Appendix). The primary objective was to compare the effect of migalastat with that of placebo on kidney GL-3 as assessed by histologic scoring of the number of inclusions in interstitial capillaries after 6 months of treatment. The secondary objectives of stage 1 were to compare the effect of migalastat with that of placebo on urinary GL-3 levels, renal function, 24-hour urinary protein excretion, and safety and adverse events. The tertiary objectives were cardiac function, patient-reported outcomes, exploratory kidney analyses, and α -galactosidase activity in white cells. Patients who completed the study were eligible to enroll in the open-label study (AT1001-041) for up to 5 years.

STUDY OVERSIGHT

The studies were approved by the institutional review board or ethics committee at each participating center and were conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All the patients provided written informed consent. A chartered data and safety monitoring board reviewed safety. The study (AT1001-011) was designed by the authors, who vouch for the completeness and accuracy of the data and analyses and for the fidelity of the study to the protocol. Data collection and analyses were undertaken by the sponsor (Amicus Therapeutics) in collaboration with a core group of investigators. The first draft of the manuscript was written by the first author and reviewed by all the authors. All the authors made the decision to submit the manuscript for publication. A full copy of the protocol and the statistical analysis plan is available at NEJM.org.

PATIENTS

Eligible patients were 16 to 74 years of age and had genetically confirmed Fabry's disease, had either never received enzyme-replacement therapy or had not received it for at least 6 months, had a mutation in the gene encoding α -galactosidase (GLA) resulting in a mutant protein that would respond to migalastat (see the following subsection), had an estimated glomerular filtration rate (GFR) of more than 30 ml per minute per 1.73 m² of body-surface area, and had a urinary GL-3 level at least four times the upper limit of the normal

range. Patients taking angiotensin-converting-enzyme inhibitors, angiotensin-receptor blockers, or renin inhibitors had to have been on stable regimens of those medications for at least 4 weeks. We assessed the baseline globotriaosylsphingosine (lyso-Gb₃) level, baseline residual α -galactosidase activity (in male patients), and number of organ systems with Fabry's disease involvement on the basis of the patients' baseline measurements and medical history (see the Methods section in the Supplementary Appendix).

DETERMINATION OF POTENTIALLY SUITABLE MUTANT α -GALACTOSIDASE

Enrollment required patients to have responsive mutant α -galactosidase forms on the basis of the assay available at the start of the study. This assay used human embryonic kidney (HEK) 293 cells into which *GLA* complementary DNA from individual patients was transfected and compared with wild-type α -galactosidase after exposure to migalastat.¹⁴ However, whether a given mutant enzyme would respond was ultimately determined by testing with an assay that became available after the study had begun, which we have termed the Migalastat "Amenability" Assay, a Good Laboratory Practice (GLP)-validated HEK assay. That assay includes several modifications to increase the quality and rigor of the test, including more rigorous plasmid DNA quality-control assessments and storage specifications (see the Methods section in the Supplementary Appendix). Testing was completed before unblinding of the stage 1 data. The criteria for potentially suitable mutant proteins were identical in the two assays. We have referred to mutations that resulted in enzymes meeting the GLP-validated HEK assay criteria as "amenable" (suitable).

ASSESSMENT OF RENAL HISTOLOGIC FEATURES

Each patient underwent a baseline kidney biopsy and repeat kidney biopsies at 6 months and 12 months. The number of GL-3 inclusions per kidney interstitial capillary per patient at baseline and at 6 and 12 months was assessed quantitatively¹⁵ in 300 capillaries by three independent pathologists who were unaware of the study medication and study visit. All values for each individual biopsy at a given time were averaged before statistical analysis. The same three pathologists qualitatively assessed GL-3 changes in podocytes, endothelial cells, and mesangial cells, as well as

glomerular sclerosis, in the same blinded fashion (see the Methods section in the Supplementary Appendix).

LYSO-GB₃ AND GL-3

Plasma lyso-Gb₃ levels and 24-hour urine GL-3 levels were analyzed by means of liquid chromatography-mass spectroscopy. This involved a new stable-isotope-labeled internal standard, ¹³C₆-lyso-Gb₃ (lower limit of quantification, 0.200 ng per milliliter, or 0.254 nmol per liter).^{16,17}

RENAL-FUNCTION ASSESSMENT

The estimated GFR was determined with the use of the Chronic Kidney Disease Epidemiology Collaboration equation,¹⁸ and the measured GFR was determined by iohexol clearance.¹⁹ Annualized rates of change from baseline were calculated. (For details, see the Methods section in the Supplementary Appendix.)

ECHOCARDIOGRAPHY

The left-ventricular-mass index, end-diastolic left ventricular posterior wall thickness, end-diastolic interventricular septum thickness, and other echocardiographic measures were assessed through blinded, centralized evaluation. The baseline visit of extension study AT1001-041 was used as the last assessment (see the Methods section in the Supplementary Appendix).

PATIENT-REPORTED OUTCOMES

Patient-reported outcomes were assessed with the use of the Gastrointestinal Symptom Rating Scale,²⁰ the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36), version 2,²¹ and the pain-severity component of the Brief Pain Inventory-Short Form.²² (For more on these assessments, see the Methods section in the Supplementary Appendix.)

SAFETY ANALYSIS AND ADVERSE EVENTS

Patients who had received at least one dose of study medication were included in the safety analysis. Safety outcomes were vital signs, physical-examination findings, electrocardiographic results, clinical laboratory measurements, and adverse events.

STATISTICAL ANALYSIS

The intention-to-treat (ITT) population consisted of the 67 randomly assigned patients. The primary

stage 1 (6-month) end point, assessed in the ITT population by means of baseline biopsy samples (in 64 patients), was the percentage of patients in the migalastat and placebo groups with a decrease of 50% or more in the number of GL-3 inclusions per kidney interstitial capillary. Two other stage 1 end points were assessed in the modified ITT population (60 randomly assigned patients with paired baseline and month 6 biopsy samples): percent change in GL-3 inclusions per kidney interstitial capillary and percent of kidney interstitial capillaries with no GL-3 inclusions. Efficacy analyses for GL-3 inclusions per kidney interstitial capillary and other prespecified end points in stage 2 (months 6 through 12) and the open-label extension (months 12 through 24) were based on the ITT population with mutant α -galactosidase enzyme shown to be suitable for migalastat treatment by the validated assay (50 patients). Changes from baseline were considered to be significant if the 95% confidence intervals did not include zero or if the P values were less than 0.05. Additional statistical methods are provided in the Supplementary Appendix.

RESULTS

BASELINE CHARACTERISTICS

A total of 67 patients (64% female) with potentially responsive mutant α -galactosidase underwent randomization (ITT population) (Fig. S1 and Table S1 in the Supplementary Appendix). There were no significant differences in baseline characteristics between the migalastat group and the placebo group among the 50 patients in the ITT population with suitable mutant α -galactosidase (Table 1).

Published reports of clinical phenotypes associated with the genotypes of the 50 patients with suitable mutations indicate that 30 (60%) had mutations associated with the classic phenotype of Fabry's disease, 1 (2%) had mutations associated with the nonclassic phenotype, 3 (6%) had mutations associated with both phenotypes, and 16 (32%) had mutations that were not yet classified (Tables S2 and S3 in the Supplementary Appendix). Residual activity of α -galactosidase in white cells of less than 3% was found in 14 of 16 male patients (87%); 29 of 31 male and female patients (94%) had elevated plasma lyso-

Gb₃ levels, and 47 of 50 male and female patients (94%) had Fabry's disease involvement in at least two organ systems (Tables S4A and S4B in the Supplementary Appendix).

GL-3 IN KIDNEY INTERSTITIAL CAPILLARIES

In the 6-month primary end-point analysis (ITT population), 13 of 32 patients (41%) who received migalastat and 9 of 32 patients (28%) who received placebo had a response ($\geq 50\%$ reduction in the number of GL-3 inclusions per kidney interstitial capillary) ($P=0.30$). The median change in interstitial capillary GL-3 from baseline was -40.8% with migalastat and -5.6% with placebo ($P=0.10$). The mean difference for the change in the percentage of interstitial capillaries with no GL-3 inclusions was 7.3 percentage points in favor of migalastat ($P=0.04$).

In stage 1 (6-month) post hoc analysis and stage 2 (12-month) prespecified analysis in 45 patients with suitable mutant α -galactosidase, 6 months of migalastat was associated with a significantly greater reduction in the mean (\pm SE) number of GL-3 inclusions per kidney interstitial capillary than was placebo: -0.25 ± 0.10 versus 0.07 ± 0.13 ; $P=0.008$ (Fig. 1, and Table S5 in the Supplementary Appendix). The reduction in interstitial capillary GL-3 at 6 months remained stable after an additional 6 months of treatment. A significant reduction in the mean number of GL-3 inclusions per kidney interstitial capillary was observed at 12 months in patients who switched from placebo to migalastat at 6 months (-0.33 ± 0.15 , $P=0.01$). Patients with mutant α -galactosidase that was not suitable for migalastat therapy according to the validated assay did not show any treatment effect in interstitial capillary GL-3 (Table S6 in the Supplementary Appendix).

GL-3 IN GLOMERULAR CELLS

On the basis of qualitative assessments of 23 kidney biopsy samples after 12 months of migalastat, patients with responsive mutant α -galactosidase showed decreases in GL-3 in glomerular podocytes (5 of 23 samples, 22%), endothelial cells (6 of 23 samples, 26%), and mesangial cells (11 of 23 samples, 48%). None of the samples had increases; the remaining samples showed no significant change.

Table 1. Baseline Characteristics of All Patients with Mutant α -Galactosidase Forms That Were Suitable for Migalastat Therapy.*

Characteristic	Double-Blind Migalastat→ Open-Label Migalastat (N=28)	Double-Blind Placebo→ Open-Label Migalastat (N=22)	Total (N=50)
Age			
No. of patients	28	22	50
Mean — yr	41.5±13.0	45.1±8.0	43.1±11.0
Median — yr	37.0	45.5	45.0
Weight			
No. of patients	28	22	50
Mean — kg	72.6±15.4	76.1±16.5	74.1±15.8
Median — kg	72.3	74.0	72.8
Time since diagnosis of Fabry's disease			
No. of patients	28	21	49
Mean — yr	5.6±6.9	7.3±8.8	6.3±7.7
Median — yr	4.1	4.1	4.1
Previous use of enzyme-replacement therapy — no. (%)†	4 (14)	7 (32)	11 (22)
Use of ACE inhibitor, ARB, or renin inhibitor at baseline — no. (%)			
Yes	9 (32)	12 (55)	21 (42)
No	19 (68)	10 (45)	29 (58)
Urinary protein excretion — no. (%)			
>150 mg/24 hr	17 (61)	18 (82)	35 (70)
>300 mg/24 hr	8 (29)	11 (50)	19 (38)
>1000 mg/24 hr	3 (11)	3 (14)	6 (12)
Measured GFR‡			
No. of patients	27	21	48
Mean — ml/min/1.73 m ²	80.0±30.9	83.1±22.8	81.3±27.5
Median — ml/min/1.73 m ²	84.9	82.2	83.4
Estimated GFR§			
No. of patients	28	22	50
Mean — ml/min/1.73 m ²	94.4±27.0	90.6±17.1	92.7±23.0
Median — ml/min/1.73 m ²	96.6	93.5	94.0
Lyso-Gb₃			
No. of patients	18	13	31
Mean — nmol/liter	47.3±62.0	41.9±39.0	45.0±53.0

* Plus-minus values are means ±SD. There were no significant differences between the two groups. Patients received 6 months of double-blind migalastat or placebo (stage 1), followed by open-label migalastat from 6 to 12 months (stage 2) plus an additional year. ACE denotes angiotensin-converting enzyme, ARB angiotensin-receptor blocker, GFR glomerular filtration rate, and lyso-Gb₃ globotriaosylsphingosine.

† Shown are patients who had received enzyme-replacement therapy more than 6 months before baseline. Patients who had received enzyme-replacement therapy within 6 months before baseline were not eligible for the study.

‡ The measured GFR was determined by iothexol clearance.¹⁹

§ The estimated GFR was determined with the use of the Chronic Kidney Disease Epidemiology Collaboration equation.¹⁸

PLASMA LYSO-GB₃ LEVELS

In the modified ITT population with suitable

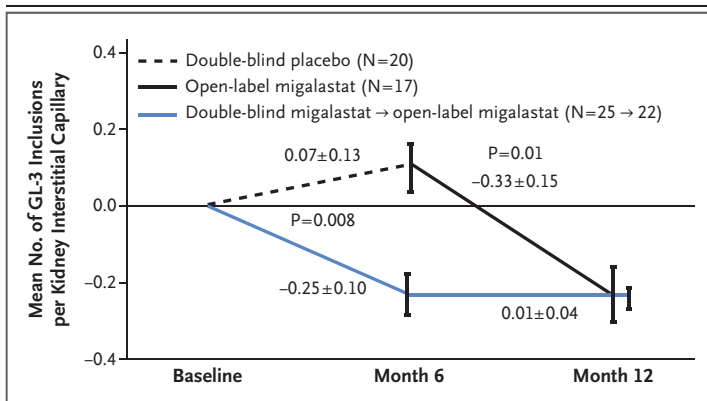


Figure 1. Change from Baseline in Kidney Interstitial Capillary Globotriaosylceramide (GL-3) in Patients with Mutant α -Galactosidase Forms That Were Suitable for Migalastat Therapy.

Baseline values were normalized to zero, and data represent the mean change from baseline or from month 6. An analysis of covariance (ANCOVA) model with covariate adjustment for baseline value and factors for treatment group and treatment-by-baseline interaction was used for the change from baseline to month 6; the P value of 0.008 corresponds to the least-squares mean difference between migalastat and placebo. A mixed-effects model for repeated measures was used for the change from month 6 to month 12 in patients switching from placebo to migalastat. The model used fixed effects for treatment group and time, time-by-treatment interaction, and baseline GL-3 inclusions. I bars indicate standard errors.

mutant α -galactosidase, 6 months of migalastat was associated with a significantly lower plasma lyso-Gb₃ level than was 6 months of placebo ($P=0.003$) (Fig. 2, and Table S7 in the Supplementary Appendix). Plasma lyso-Gb₃ levels remained stable without further reduction after 6 additional months of migalastat. In patients switching from placebo to migalastat at 6 months, a significant reduction in plasma lyso-Gb₃ levels was observed between month 6 and month 12 ($P<0.001$) (Fig. 2, and Table S7 in the Supplementary Appendix). Plasma levels in patients with mutant α -galactosidase that was not suitable for migalastat therapy were unchanged (Table S8 in the Supplementary Appendix).

URINARY GL-3 SUBSTRATE

In patients with suitable mutant α -galactosidase, the mean (\pm SE) change in the 24-hour urinary GL-3 substrate concentration from baseline to month 6 was -361 ± 169 ng per milligram of creatinine with migalastat (concentration at month 6, 555 ± 151) and -147 ± 217 ng per milligram of creatinine with placebo (concentration at month 6, 1017 ± 218) ($P=0.44$).

KIDNEY FUNCTION

In the modified ITT population with suitable mutant α -galactosidase, there were no significant between-group differences in the change in estimated GFR or measured GFR from baseline to month 6 (see the Results section in the Supplementary Appendix). In patients followed for up to 24 months of migalastat, the mean (\pm SE) annualized changes from baseline in estimated GFR and measured GFR (\pm SE) were -0.30 ± 0.66 and -1.51 ± 1.33 ml per minute per 1.73 m², respectively. Male sex and a higher 24-hour urinary protein excretion at baseline were associated with a higher rate of annual decline (Table S9 in the Supplementary Appendix). There were no significant differences in baseline levels or changes from baseline between study groups for 24-hour urinary protein excretion (Table S10 in the Supplementary Appendix).

ECHOCARDIOGRAPHIC VARIABLES

At baseline, the left-ventricular-mass index was similar in the migalastat and placebo groups; there were no significant differences during stage 1 (Tables S11 and S12 in the Supplementary Appendix). In patients in the ITT population with

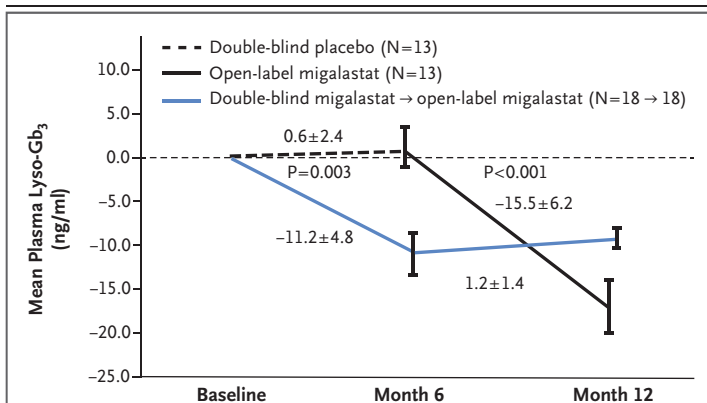


Figure 2. Change from Baseline in Plasma Globotriaosylsphingosine (Lyso-Gb₃) Levels in Patients with Suitable Mutant α -Galactosidase.

Baseline values were normalized to zero, and data represent the mean change from baseline or from month 6. An ANCOVA model was used to compare migalastat with placebo from baseline to month 6 and to compare the change from month 6 to month 12 in patients switching from placebo to migalastat. The ANCOVA model used covariate adjustment for baseline value and factors for treatment group and treatment-by-baseline interaction. P values correspond to the least-squares mean difference between migalastat and placebo. Of the 44 patients who consented to the plasma lyso-Gb₃ analyses, 31 had suitable mutant α -galactosidase. I bars indicate standard errors.

Table 2. Changes in Echocardiographic-Derived Left-Ventricular-Mass (LVM) Index from Baseline to Month 18 or 24.*

Population	LVM Index at Baseline	Change in LVM Index from Baseline to Month 18 or 24†
	g/m ²	g/m ² (95% CI)
Patients with suitable mutant α -galactosidase		
No. of patients	44	27
Mean value	96.5±5.0	-7.7±3.7 (-15.4 to -0.01)‡
Patients with suitable mutant α -galactosidase and LVH at baseline		
No. of patients	11	8
Mean value	138.9±11.0	-18.6±8.3 (-38.2 to 1.0)

* Plus-minus values are means \pm SE. Shown are patients in the intention-to-treat population who had suitable mutant α -galactosidase, underwent baseline and postbaseline echocardiography, and received migalastat for at least 18 months. Month 6 was used as the baseline for patients who received placebo for 6 months before switching to migalastat. The normal range for the LVM index is 43 to 95 g per square meter in women and 49 to 115 g per square meter in men. CI denotes confidence interval, and LVH left ventricular hypertrophy.

† Month 18 or 24 was used as the baseline of the extension study.

‡ The change from baseline was considered to be significant because the 95% confidence interval did not include zero.

suitable mutant α -galactosidase who received migalastat for up to 24 months, a significant decrease in the left-ventricular-mass index was observed overall, with a trend toward a larger reduction in patients with left ventricular hypertrophy at baseline (Table 2). The annualized rate of change from baseline to month 24 is provided in Table S13 in the Supplementary Appendix.

The end-diastolic interventricular septum thickness changed by -0.06 ± 0.05 cm (95% confidence interval, -1.67 to 0.04) from the baseline value of 1.17 ± 0.06 cm, representing a decrease of 5.2%; the end-diastolic left ventricular posterior wall thickness was stable for up to 24 months (Table S14 in the Supplementary Appendix). The changes in the left-ventricular-mass index correlated with changes in the intraventricular septum thickness ($R^2=0.26$, $P=0.006$) but not with changes in the left ventricular posterior wall thickness ($R^2=0.06$, $P=0.23$).

GASTROINTESTINAL SYMPTOMS

Among migalastat-treated patients in the ITT population with suitable mutant α -galactosidase, symptoms decreased in three domains (diarrhea, reflux, and indigestion) of five domains in the Gastrointestinal Symptom Rating Scale (Table S15 in the Supplementary Appendix). For the diarrhea domain, between baseline and month 6 (stage 1), there was a significant decrease in symptoms ($P=0.03$); a nonsignificant decrease was

observed among patients who had symptoms at baseline ($P=0.06$). Significant changes over a period of 24 months were observed for patients with or without baseline symptoms.

There was a significant improvement in the reflux domain during stage 1 in patients in the ITT population with suitable mutant α -galactosidase who had baseline symptoms ($P=0.047$). Significant changes over 24 months were observed in the indigestion domain for patients with or without baseline symptoms. There was a trend toward improvement in the constipation domain. Results on the SF-36 and the pain-severity component of the Brief Pain Inventory–Short Form are provided in the Results section in the Supplementary Appendix.

SAFETY AND ADVERSE EVENTS

During stage 1, adverse events that emerged after the initiation of a study medication were similar in the migalastat and placebo groups. Adverse events with a higher frequency among patients receiving migalastat than among those receiving placebo were headache (12 of 34 patients [35%] vs. 7 of 33 patients [21%]) and nasopharyngitis (6 of 34 patients [18%] vs. 2 of 34 patients [6%]). The most frequently reported adverse events during stage 2 were headache (9 of 63 patients [14%]) and procedural pain (7 of 63 patients [11%] related to kidney biopsies) during stage 2 and proteinuria (9 of 57 patients [16%]), headache

Table 3. Severe Adverse Events That Emerged after the Initiation of a Study Medication (Stage 1).

Preferred Term	Migalastat (N = 34)		Placebo (N = 33)	
	No. (%) [*]	Relationship to Treatment [†]	No. (%) [*]	Relationship to Treatment [†]
Headache	2 (6)	Unrelated	0	Not applicable‡
Hematuria	1 (3)	Unrelated	0	Not applicable‡
Hydronephrosis	1 (3)	Unrelated	0	Not applicable‡
Menorrhagia	1 (3)	Unrelated	0	Not applicable‡
Viral meningitis	0	Not applicable‡	1 (3)	Unrelated
Overdose of study medication	0	Not applicable‡	1 (3)	Unrelated

* Percentages were calculated on the basis of the total number of patients treated with the same dose and regimen.

† Relationship to treatment was determined by the principal investigator at the study site.

‡ No severe events with this preferred term occurred in this study group.

(6 of 57 patients [11%]), and bronchitis (6 of 57 patients [11%]) during the open-label extension. Most adverse events were mild or moderate in severity (Table 3). No adverse events led to the discontinuation of migalastat. Additional safety data are provided in Tables S16 through S18 in the Supplementary Appendix.

Serious adverse events were reported in 7 patients during stage 1 (5 in the migalastat group and 2 in the placebo group), 5 during stage 2, and 11 during the open-label extension. Two serious adverse events were assessed by the investigator as being possibly related to migalastat: fatigue and paresthesia. Both occurred in the same patient between months 12 and 24, and both resolved. No individual serious adverse event was reported by more than 1 patient. Two patients discontinued migalastat owing to serious adverse events; both events were deemed to be unrelated to migalastat. No deaths were reported. All serious adverse events and their possible association with a study medication are provided in Table 4.

Proteinuria that emerged after the initiation of a study medication was reported in 9 of 57 patients (16%) between months 12 and 24; one case of proteinuria was judged to be migalastat-related. In 5 patients, the 24-month values were in the same range as the baseline values. Three patients with suitable mutant α -galactosidase had overt proteinuria (urinary protein excretion of >1 g per 24 hours) at baseline, which worsened over a period of 24 months. In 23 of 28 patients who had a urinary protein excretion of 300 mg or less per 24 hours at baseline, the urinary protein ex-

cretion remained stable during migalastat treatment (Table S19 in the Supplementary Appendix).

No patients had progression to end-stage renal disease, cardiac death, or stroke, as defined by Banikazemi et al.²³ There was a single case of transient ischemic attack, which was judged to be unrelated to migalastat. Analyses of vital signs, physical-examination findings, clinical laboratory measurements, and electrocardiographic results did not reveal any clinically relevant effect of migalastat.

DISCUSSION

Migalastat, the oral pharmacologic chaperone administered in this study, has a unique mechanism of action. The stabilization of suitable mutant forms of α -galactosidase by migalastat is hypothesized to increase enzyme levels more consistently than enzyme-replacement therapy given every 2 weeks. The higher volume of distribution of migalastat than of enzyme replacement¹¹ suggests that migalastat may enhance α -galactosidase levels in multiple organs, including the central nervous system.⁴ Migalastat, a low-molecular-weight iminosugar, would be expected to have a low burden of treatment²⁴ and would avoid the risk of the immunogenicity reactions and complications associated with enzyme-replacement therapy.¹⁰

The accumulation of GL-3 in different kidney cells is a known consequence of Fabry's disease.¹ Plasma lyso-Gb₃ has become recognized as an important marker of disease severity.^{25,26} Al-

though the primary analysis for interstitial capillary GL-3 in stage 1 (baseline to month 6) in patients with mutant α -galactosidase forms that were suitable or not suitable for migalastat therapy did not show a significant treatment effect, post hoc stage 1 and prespecified stage 2 (month 6 through 12) analyses in patients with suitable mutations provide evidence of a significant and durable reduction in kidney GL-3 levels. Tertiary analyses in this population also indicated that migalastat treatment was associated with reductions in plasma lyso-Gb₃ levels. Using a qualitative method, we found substrate reductions in other types of kidney cells. No changes in substrate levels were observed in samples from patients with mutant α -galactosidase forms that were not suitable for migalastat therapy. The clear difference in effect on substrate in patients with suitable versus unsuitable mutations supports the accuracy and predictive value of the GLP-validated HEK assay in identifying patients with GLA mutations who will have a response to migalastat.

Progressive decline in renal function is a major complication of Fabry's disease.^{27,28} In stage 1 (baseline to month 6), there was no significant difference in the annualized rate of change in GFR between patients who received migalastat and those who received placebo; this observation period, however, was not sufficiently long to assess differences in GFR between groups. After up to 24 months (prespecified end point in stage 2 and open-label extension), the annualized rates of change in estimated GFR among migalastat-treated patients with amenable mutations at all baseline levels of urinary protein excretion were less than the decline in estimated GFR in published cohorts of untreated patients.^{28,29} We balanced the baseline characteristics of patients in our study with those of the published cohorts for sex, renal function, and urinary protein excretion. Other variables not reported in the published cohorts (e.g., kidney GL-3) may have differed, thus limiting the comparisons. Male sex and higher urinary protein excretion are key predictive factors for more rapid progression of Fabry nephropathy, frequently resulting in dialysis or end-stage renal disease.³⁰ The changes that we observed in 24-hour urinary protein excretion were similar to those reported for patients treated with enzyme-replacement therapy.³¹ Our results suggest that treatment with migalastat, as compared with

Table 4. Serious Adverse Events That Emerged after the Initiation of a Study Medication (24 Months).

Treatment Group and Preferred Term	Relationship to Treatment*
Double-blind migalastat→open-label migalastat	
Malaise	Unrelated
Postprocedural hematoma	Unrelated
Bulimia nervosa	Unrelated
Amyotrophic lateral sclerosis	Unrelated
Cerebral hemorrhage	Unrelated
Pulmonary embolism	Unrelated
Pneumothorax	Unrelated
Hydronephrosis	Unrelated
Palpitations	Unrelated
Ventricular tachycardia	Unrelated
Constipation	Unrelated
Transient ischemic attack	Unrelated
Double-blind placebo→open-label migalastat	
Fatigue	Possibly related
Paresthesia	Possibly related
Bone cyst	Unrelated
Anaplastic large-cell lymphoma, T-cell and null-cell types	Unrelated
Syncope	Unrelated
Lower abdominal pain	Unrelated
Pulmonary embolism	Unrelated
Deep-vein thrombosis	Unrelated
Postprocedural hemorrhage	Unrelated
Noncardiac chest pain	Unrelated
Viral meningitis	Unrelated
Multiple fractures	Unrelated
Helicobacter gastritis	Unrelated
Bacterial infection	Unrelated

* Relationship to treatment was determined by the principal investigator at the study site.

no treatment, improved renal function in both male and female patients with Fabry's disease at all levels of urinary protein excretion.

Cardiac complications are common and the main cause of death in Fabry's disease.³² The most frequent cardiac manifestation is left ventricular hypertrophy,³³ an important risk factor for cardiac events.^{34,35} Reduction of left ventricular mass has been shown to improve outcomes in these patients.³⁶ Migalastat treatment for up to 24 months significantly reduced the left-ventricu-

lar-mass index, with larger decreases observed in patients with left ventricular hypertrophy at baseline. These findings suggest that the effects of migalastat on the left-ventricular-mass index compare favorably with those observed for enzyme-replacement therapy; the effect of enzyme-replacement therapy on left ventricular mass may be inconsistent or diminish over time.^{33,35,36} This reduction in the left-ventricular-mass index by migalastat may contribute to a decrease in cardiac complications that are common in Fabry's disease,³³⁻³⁵ but further study is warranted.

Patients with Fabry's disease frequently have debilitating gastrointestinal symptoms.^{37,38} Six months of double-blind migalastat was associated with improvement in the diarrhea domain of the Gastrointestinal Symptom Rating Scale as compared with placebo. Improvements were seen in several of the domains over 24 months. These migalastat-associated improvements may have a

long-term positive effect on quality of life, but this observation requires further study.

There were no discontinuations of migalastat owing to adverse events related to migalastat during the study. No patients had progression to end-stage renal disease, had strokes, or died from cardiac causes during the study.

In conclusion, among patients with mutant α -galactosidase forms that were suitable or not suitable for migalastat therapy, the percentage of patients who had a decrease of 50% or more in the number of GL-3 inclusions per kidney interstitial capillary at 6 months did not differ significantly between the migalastat group and the placebo group.

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APPENDIX

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REFERENCES

1. Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease — ceramidetrihexosidase deficiency. *N Engl J Med* 1967;276:1163-7.
2. Germain DP. Fabry disease. *Orphanet J Rare Dis* 2010;5:30.
3. Fan JQ, Ishii S, Asano N, Suzuki Y. Accelerated transport and maturation of lysosomal α -galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. *Nat Med* 1999;5:112-5.
4. Khanna R, Soska R, Lun Y, et al. The pharmacological chaperone 1-deoxygalactonojirimycin reduces tissue globotriaosylceramide levels in a mouse model of Fabry disease. *Mol Ther* 2010;18:23-33.
5. Benjamin ER, Flanagan JJ, Schilling A, et al. The pharmacological chaperone 1-deoxygalactonojirimycin increases α -galactosidase A levels in Fabry pa-

- tient cell lines. *J Inherit Metab Dis* 2009;32:424-40.
6. Yam GH, Zuber C, Roth J. A synthetic chaperone corrects the trafficking defect and disease phenotype in a protein misfolding disorder. *FASEB J* 2005;19:12-8.
 7. Germain DP, Fan JQ. Pharmacological chaperone therapy by active-site-specific chaperones in Fabry disease: in vitro and preclinical studies. *Int J Clin Pharmacol Ther* 2009;47:Suppl 1:S111-7.
 8. Wilcox WR, Linthorst GE, Germain DP, et al. Anti- α -galactosidase A antibody response to agalsidase beta treatment: data from the Fabry Registry. *Mol Genet Metab* 2012;105:443-9.
 9. Bénichou B, Goyal S, Sung C, Norfleet AM, O'Brien F. A retrospective analysis of the potential impact of IgG antibodies to agalsidase β on efficacy during enzyme replacement therapy for Fabry disease. *Mol Genet Metab* 2009;96:4-12.
 10. Lenders M, Stypmann J, Duning T, Schmitz B, Brand SM, Brand E. Serum-mediated inhibition of enzyme replacement therapy in Fabry disease. *J Am Soc Nephrol* 2016;27:256-64.
 11. Johnson FK, Mudd PN Jr, Bragat A, Adera M, Boudes P. Pharmacokinetics and safety of migalastat HCl and effects on agalsidase activity in healthy volunteers. *Clin Pharmacol Drug Dev* 2013;2:120-32.
 12. Genzyme Therapeutics. Summary of product characteristics: Fabrazyme 35 mg, powder for concentrate for solution for infusion. 2014 (<http://www.medicines.org.uk/emc/medicine/18404/SPC/Fabrazyme+35+mg,+powder+for+concentrate+for+solution+for+infusion/>).
 13. Shire Human Genetic Therapies. Summary of product characteristics: Replagal 1mg/ml concentrate for solution for infusion. 2014 (<https://www.medicines.org.uk/emc/medicine/19760/SPC/Replagal+1mg+ml+concentrate+for+solution+for+infusion/>).
 14. Wu X, Katz E, Della Valle MC, et al. A pharmacogenetic approach to identify mutant forms of α -galactosidase A that respond to a pharmacological chaperone for Fabry disease. *Hum Mutat* 2011;32:965-77.
 15. Barisoni L, Jennette JC, Colvin R, et al. Novel quantitative method to evaluate globotriaosylceramide inclusions in renal peritubular capillaries by virtual microscopy in patients with Fabry disease. *Arch Pathol Lab Med* 2012;136:816-24.
 16. Boutin M, Auray-Blais C. Multiplex tandem mass spectrometry analysis of novel plasma lyso-Gb₃-related analogues in Fabry disease. *Anal Chem* 2014;86:3476-83.
 17. Benjamin ER, Hamler R, Brignol N, et al. Migalastat reduces plasma globotriaosylsphingosine (lyso-Gb₃) in Fabry patients: results from the FACETS phase 3 study. *J Inherit Metab Dis* 2014;37:S161. abstract.
 18. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;150:604-12.
 19. Nilsson-Ehle P. Iohexol clearance for the determination of glomerular filtration rate: 15 years' experience in clinical practice. *EJIFCC* 2001;13(2):1-5.
 20. Svedlund J, Sjödin I, Dotevall G. GSRS — a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988;33:129-34.
 21. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992;30:473-83.
 22. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore* 1994;23:129-38.
 23. Banikazemi M, Bultas J, Waldek S, et al. Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Ann Intern Med* 2007;146:77-86.
 24. Germain DP, Giugliani R, Hughes DA, et al. Safety and pharmacodynamic effects of a pharmacological chaperone on α -galactosidase A activity and globotriaosylceramide clearance in Fabry disease: report from two phase 2 clinical studies. *Orphanet J Rare Dis* 2012;7:91.
 25. Rombach SM, Dekker N, Bouwman MG, et al. Plasma globotriaosylsphingosine: diagnostic value and relation to clinical manifestations of Fabry disease. *Biochim Biophys Acta* 2010;1802:741-8.
 26. van Breemen MJ, Rombach SM, Dekker N, et al. Reduction of elevated plasma globotriaosylsphingosine in patients with classic Fabry disease following enzyme replacement therapy. *Biochim Biophys Acta* 2011;1812:70-6.
 27. Pisani A, Visciano B, Imbriaco M, et al. The kidney in Fabry's disease. *Clin Genet* 2014;86:301-9.
 28. Schiffmann R, Warnock DG, Banikazemi M, et al. Fabry disease: progression of nephropathy, and prevalence of cardiac and cerebrovascular events before enzyme replacement therapy. *Nephrol Dial Transplant* 2009;24:2102-11.
 29. Wanner C, Oliveira JP, Ortiz A, et al. Prognostic indicators of renal disease progression in adults with Fabry disease: natural history data from the Fabry Registry. *Clin J Am Soc Nephrol* 2010;5:2220-8.
 30. Waldek S, Feriozzi S. Fabry nephropathy: a review — how can we optimize the management of Fabry nephropathy? *BMC Nephrol* 2014;15:72.
 31. Warnock DG, Ortiz A, Mauer M, et al. Renal outcomes of agalsidase beta treatment for Fabry disease: role of proteinuria and timing of treatment initiation. *Nephrol Dial Transplant* 2012;27:1042-9.
 32. Waldek S, Patel MR, Banikazemi M, Lemay R, Lee P. Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry. *Genet Med* 2009;11:790-6.
 33. Weidemann F, Niemann M, Warnock DG, Ertl G, Wanner C. The Fabry cardiomyopathy: models for the cardiologist. *Annu Rev Med* 2011;62:59-67.
 34. Patel MR, Cecchi F, Cizmarik M, et al. Cardiovascular events in patients with Fabry disease natural history data from the Fabry Registry. *J Am Coll Cardiol* 2011;57:1093-9.
 35. Germain DP, Weidemann F, Abiose A, et al. Analysis of left ventricular mass in untreated men and in men treated with agalsidase- β : data from the Fabry Registry. *Genet Med* 2013;15:958-65.
 36. Weidemann F, Niemann M, Breunig F, et al. Long-term effects of enzyme replacement therapy on Fabry cardiomyopathy: evidence for a better outcome with early treatment. *Circulation* 2009;119:524-9.
 37. Hoffmann B, Schwarz M, Mehta A, Keshav S. Gastrointestinal symptoms in 342 patients with Fabry disease: prevalence and response to enzyme replacement therapy. *Clin Gastroenterol Hepatol* 2007;5:1447-53.
 38. Banikazemi M, Ullman T, Desnick RJ. Gastrointestinal manifestations of Fabry disease: clinical response to enzyme replacement therapy. *Mol Genet Metab* 2005;85:255-9.

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