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ARTICLE

Long-term safety and efficacy of pegunigalsidase alfa: A multicenter 6-year study in adult patients with Fabry disease

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ABSTRACT

Purpose: Fabry disease (FD) is a rare lysosomal storage disorder caused by pathogenic variants in the *GLA* gene encoding α-galactosidase (α-Gal)-A. We evaluated long-term safety/efficacy of pegunigalsidase alfa, a novel PEGylated α-Gal-A enzyme replacement therapy (ERT) now approved for FD.

Methods: In a phase-1/2 dose-ranging study, 15 ERT-naive adults with FD completed 12 months of pegunigalsidase alfa and enrolled in this 60-month open-label extension of 1 mg/kg pegunigalsidase alfa infusions every 2 weeks.

Results: Fifteen patients enrolled (8 males; 7 females); 10 completed ≥48 months (60 months total treatment), and 2 completed 60 months (72 months total treatment). During treatment, most treatment-emergent adverse events were mild/moderate in severity and all infusion-related reactions were mild/moderate in severity. Four patients were transiently positive for anti-pegunigalsidase alfa IgG. Patients showed continuous reduction in plasma lyso-Gb3 concentrations with mean (standard error) reduction of 76.1 [25.1] ng/mL from baseline to month 24. At 60 months, the estimated glomerular filtration rate slope was comparable to that observed in patients treated with other ERTs. Cardiac function assessments revealed stability; no cardiac fibrosis was observed.

Conclusion: In this first long-term assessment of pegunigalsidase alfa administration in patients with FD, we found favorable safety/efficacy. Our data suggest long-term continuous benefits of pegunigalsidase alfa treatment in adults with FD.

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Introduction

Fabry disease (FD) is an inherited, X-linked, progressive, lysosomal storage disorder.[1] Although FD is considered rare, with previous reports approximating its incidence at approximately 1 in 40,000 males, recent newborn screening initiatives have found a higher incidence of the disease (approximately 1 in 3100 male newborns in Italy and 1 in 1500 males in Taiwan), indicating the true prevalence might be higher than previously thought.[2-4]

FD is caused by pathogenic variants in the *GLA* gene encoding the lysosomal enzyme α-galactosidase (α-Gal)-A, which results in deficient or absent levels of enzyme activity.[1] The classical FD phenotype, defined by very low or complete lack of α-Gal-A activity, is characterized by early symptom onset, multiorgan involvement, aggressive disease course, and reduced life expectancy; males with this phenotype have the highest rate of Fabry-related clinical events.[5] The non-classic FD phenotype, defined by higher residual α-Gal-A activity, is characterized by later onset of symptoms and a milder disease course that is often confined to 1 organ. However, some non-classic patients with FD may develop more advanced symptoms of disease including organ damage.[6] Heterozygous female patients present with a wide spectrum of disease severity ranging from asymptomatic to severe, possibly because of skewed X-inactivation.[7]

In FD, lower residual α-Gal-A enzyme activity leads to accumulation of increasingly toxic levels of glycosphingolipids, primarily globotriaosylceramide (Gb3) and its derivative globotriaosylsphingosine (lyso-Gb3), which affect the kidneys, heart, and nervous system.[1,8] This can greatly affect quality of life as 60% to 80% of patients with classic FD experience pain, described as either episodic crises characterized by agonizing burning or chronic pain characterized by burning and tingling.[8] Damage caused to these organ systems can also ultimately progress to organ failure, thereby limiting life expectancy.[8]

Enzyme replacement therapies (ERTs) are commercially available for the treatment of FD in several countries: agalsidase alfa (Replagal, Takeda Pharmaceuticals International AG)[9] and agalsidase beta (Fabrazyme, Genzyme Corporation [a Sanofi company]).[10] The introduction of these ERTs has resulted in substantial improvements in the course of the disease; nevertheless, disease progression cannot be fully halted and can be further affected by the occurrence of anti-drug antibodies (ADAs) and infusion-related reactions (IRRs).[10] Although agalsidase alfa carries a lower risk of ADAs and IRRs, it delivers a lower dose of enzyme.[9,10] Neutralizing ADAs have been seen in 40% to 77% of adult patients treated with either agalsidase alfa or agalsidase beta, and cross-reactivity of these neutralizing ADAs has been noted.[10,11] Development of neutralizing antibodies is associated with lower therapeutic effectiveness.[11-13]

Abbreviations

α-Gal – α-galactosidase
ADA – anti-drug antibody
BPI-SF – Short-Form Brief Pain Inventory
CKD-EPI – Chronic Kidney Disease-Epidemiology Collaboration
COPD – chronic obstructive pulmonary disease
eGFR – estimated glomerular filtration rate
ERT – enzyme replacement therapy
FD – Fabry disease
Gb3 – globotriaosylceramide
GSA – Gastrointestinal Symptoms Assessment
IgG – immunoglobulin G
IRR – infusion-related reaction
KDIGO – Kidney Disease: Improving Global Outcomes
LVEF – left ventricular ejection fraction
LVM – left ventricular mass
LVMI – left ventricular mass index
Lyso-Gb3 – globotriaosylsphingosine
MSSI – Mainz Severity Score Index
PEG – polyethylene glycol
SE – standard error
TEAE – treatment-emergent adverse event
UPCR – urine protein to creatinine ratio

Pegunigalsidase alfa is a novel PEGylated α-Gal-A ERT recently approved in the European Union and United States for the treatment of FD.[14,15] Because of the PEGylation, pegunigalsidase alfa has a prolonged circulatory half-life (approximately 80 hours)[9,10,16,17] compared with other currently available ERTs (~≤2 hours).[9,10] Theoretically, PEGylation of the enzyme may also contribute to decreased immunogenicity.[16,17] Accordingly, in vitro studies have shown that ADAs from some patients who had received agalsidase alfa or agalsidase beta have lower affinity and enzyme inhibition for pegunigalsidase alfa than for agalsidase alfa or agalsidase beta.[18] In a phase 1/2 clinical trial, pegunigalsidase alfa administered for 1 year to adult ERT-naive patients with FD had relatively low immunogenicity, with only 3 patients (19%) developing ADAs.[17] In 2 of these patients, the ADAs were transiently positive for neutralizing activity, both becoming non-neutralizing as treatment continued. The immunogenicity profile of pegunigalsidase alfa will need to be further assessed in the real-world setting.

Pegunigalsidase alfa has been well tolerated and has demonstrated meaningful impact on clinical outcomes. In ERT-naive patients, pegunigalsidase alfa treatment resulted in decreased Gb3 kidney deposition, decreased plasma lyso-Gb3, and reduced decline of kidney function,[17] indicating that pegunigalsidase alfa has clinical benefit in patients with FD treated up to 1 year.

Here, we report on the long-term safety and efficacy of pegunigalsidase alfa in adult patients with FD who received treatment for up to 72 months.

Materials and Methods

Study population

Eligibility for inclusion in studies F01/02 (PB-102-F01 and PB-102-F02, NCT01678898) has been previously described.[17] Briefly, adults ≥18 years old with confirmed FD who were ERT-naive or had not received ERT in the previous 6 months were eligible. Patients were eligible to enroll in study F03 (PB-102-F03, NCT01981720) if they had completed both F01/02.

Study design

The design of the 3-month dose-ranging study F01 ($N = 18$) and its 9-month extension F02 ($N = 16$) have been previously described.[17] In these studies, participants received intravenous infusions of pegunigalsidase alfa every 2 weeks (±3 days) at doses of either 0.2, 1, or 2 mg/kg. The second extension study, F03 ($N = 15$) was a phase 1/2, open-label, multinational, multicenter, 60-month extension study offering all patients the option to receive a total of up to 72 months of treatment (Figure 1). In this study, the target dose for all participants was 1 mg/kg. Therefore, for patients who had previously received 0.2 mg/kg pegunigalsidase alfa, the dose was gradually increased by 0.2 mg/kg at every third infusion up to 1 mg/kg. For patients who previously received 2 mg/kg pegunigalsidase alfa, the dose was decreased by 0.25 mg/kg at every third infusion down to 1 mg/kg.

In study F01, infusion durations started at ≥4 hours. If the first 4 infusions were well tolerated, the infusion duration was shortened by 2 hours. The infusion rate was then further adjusted according to the patient's tolerability: if infusions were well tolerated, the duration was shortened by 30 minutes every third infusion to a minimum of 1.5 hours.

Safety

The primary endpoint of F03 was the number of patients who experienced a treatment-emergent adverse event (TEAE), including IRRs, which were defined as TEAEs occurring during or within 2 hours after completion of an infusion. Severity of TEAEs was assessed using the Common Terminology Criteria for Adverse Events, version 4.03.

Patients were also assessed for development of anti-pegunigalsidase immunoglobulin G (IgG). Presence, titers, and neutralizing activity of ADAs were determined using either a solid-phase enzyme-linked immunosorbent assay or an in vitro enzymatic activity procedure. These assays were developed and validated based on the current immunogenicity guidelines from the US Food and Drug Administration and the European Medicines Agency.

Due to COVID-19 restrictions, 1 patient was treated for longer (75.5 months) than the total protocol-specified 72-month treatment period.

Clinical effect

Plasma lyso-Gb3 and Gb3 concentrations and kidney function were assessed every 3 months up to month 24 and every 6 months thereafter. Kidney function was assessed by estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation,[19] and by proteinuria, as determined from a spot urine sample and expressed as the urine protein to creatinine

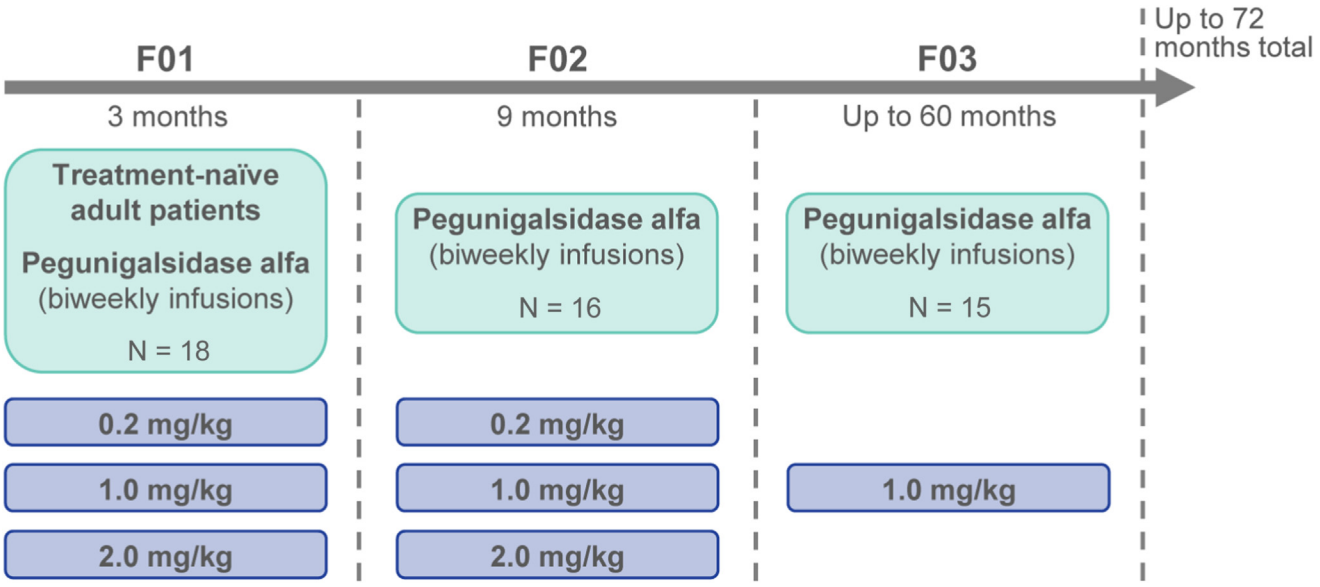

Figure 1 **Study design.** The maximum overall duration of treatment with pegunigalsidase alfa was 72 months: 3 months in F01, 9 months in F02, and up to 60 months in F03. Planned evaluation visits occurred ± 6 days at months 2 and 3, every 3 months to month 24, then every 6 months up to month 60. A final visit occurred at 3 months after the last infusion (± 6 days).

ratio (UPCR). UPCR was classified into 3 categories per kidney disease: improving global outcomes (KDIGO) guidance: normal to mildly increased (<150 mg/g), moderately increased (150-500 mg/g), and severely increased (>500 mg/g).[20]

Left ventricular mass (LVM), LVM index (LVMI), left ventricular ejection fraction (LVEF), and myocardial fibrosis were assessed by cardiac magnetic resonance imaging every 12 months. The Mainz Severity Score Index (MSSI) was used to assess the overall burden of FD at baseline and every 6 months thereafter.

Patient-reported outcomes were collected using the short-form brief pain inventory (BPI-SF) and the gastrointestinal symptoms assessment (GSA) questionnaire, which were collected every 3 months up to month 24 and every 6 months thereafter.

Analysis

Because of the small number of patients, only descriptive statistics (mean ± standard errors [SE]) are presented unless otherwise stated. All enrolled patients were included in both the safety and efficacy populations.

Results

Patients

Of the 16 patients with FD who completed study F02, 15 (7 female and 8 male) continued to study F03. Five of these patients (33.3%) discontinued prematurely: 1 due to death unrelated to study treatment and 4 owing to voluntary withdrawal. Reasons for voluntary withdrawal include pregnancy and/or planning for pregnancy ($n = 2$), financial burden associated with traveling to the study site ($n = 1$), and caregiver decision to withdraw a patient ($n = 1$). The death occurred after 39 months of treatment and the voluntary withdrawals occurred after 14 ($n = 2$) and 15 ($n = 2$) months of treatment. Ten patients (4 female and 6 male; 66.7%) completed study F03, defined as receiving a total of ≥48 months of treatment with pegunigalsidase alfa, and enrolled in a further extension study in which they are continuing to receive 1 mg/kg pegunigalsidase alfa every 2 weeks for up to an additional 60 months (study F60; NCT03566017).

Baseline demographics and characteristics at the time of enrollment in study F01 for the 15 patients who continued on to study F03 are presented in Table 1. Mean (median) age at enrollment was 33.4 (32.0) years (range: 17-54) and most patients were White ($n = 12$; 80.0%). Analysis of patient genetic data revealed that out of the 13 pathogenic *GLA* (HGNC:4296) variants observed in this study, c.400T>G and c.803_806del were the most common, each observed in 2 patients (Supplemental Table 1). Patient α-Gal-A activity was measured at baseline of the published F01 study and is included in Supplemental Table 1.[17] At study completion (05 August 2021), the cumulative duration of treatment was >12 months for all 15 patients (100.0%), ≥36 months for 11 patients (73.3%), and ≥60 months for 10 patients (66.7%), with 2 (13.3%) patients reaching 72 months of reportable data.

Safety

During the overall treatment period (baseline of F01 to last visit of F03), all 15 patients reported at least 1 TEAE. Of the 440 total TEAEs, about half (224/440 [50.9%]) occured during F03. Most (97.5%) TEAEs were mild or moderate in severity and resolved over time (Table 2). The most common TEAEs were fatigue (8 of 15 patients [53.3%]), back pain (6 of 15 patients [40.0%]), nausea, upper respiratory tract infection, nasopharyngitis, headache, paresthesia, vomiting, rash, and cough (5 of 15 patients each [33.3%]), and abdominal pain (4 of 15 patients [26.7%]). All TEAEs that were defined as severe (5 of 15 patients [33.3%]) and serious (3 of 15 patients [20.0%]) occurred in males. Serious TEAEs reported during the overall treatment period were pneumonia, clavicle fracture, perirenal hematoma, and chronic obstructive pulmonary disease (COPD), each seen in 1 patient (6.7%). None were considered related to study treatment. The events of pneumonia and COPD occurred in the same patient, with exacerbation of COPD leading to death. The treating physician considered COPD to be due to

Table 1 Baseline characteristics at study F01 entry

	Duration of Treatment[a]			
	> 12 Months	≥ 36 Months	≥ 60 Months	72 Months
Category	$N = 15$	$n = 11$	$n = 10$	$n = 2$
Median age, years (range)	32.0 (17-54)	33.0 (20-54)	30.5 (20-54)	30.0 (26-34)
Sex, *n* (%)				
Men	8 (53.3)	7 (63.6)	6 (60.0)	1 (50.0)
Women	7 (46.7)	4 (36.4)	4 (40.0)	1 (50.0)
Race, *n* (%)				
White	12 (80.0)	10 (90.9)	9 (90.0)	1 (50.0)
Black	3 (20.0)	1 (9.1)	1 (10.0)	1 (50.0)

[a]Treatment duration refers to the total duration in the overall treatment period (studies F01/F02/F03) with reportable data.

Table 2 TEAEs and IRRs

	Duration of Treatment[a]							
	> 12 Months		≥ 36 Months		≥ 60 Months		72 Months	
	N = 15		*n* = 11		*n* = 10		*n* = 2	
Category, n (%)	Patients	Events	Patients	Events	Patients	Events	Patients	Events
At least 1 TEAE	15 (100.0)	440	11 (100.0)	387	10 (100.0)	352	2 (100.0)	70
At least 1 mild or moderate TEAE[b]	15 (100.0)	429 (97.5)	11 (100.0)	376 (97.2)	10 (100.0)	342 (97.2)	2 (100.0)	70 (100.0)
At least 1 severe TEAE[b]	5 (33.3)	11 (2.5)	5 (45.5)	11 (2.8)	4 (40.0)	10 (2.8)	0	0
At least 1 SAE	3 (20.0)	4 (0.9)	3 (27.3)	4 (1.0)	2 (20.0)	2 (0.6)	0	0
At least 1 related TEAE[b]	9 (60.0)	59[c] (13.4)	6 (54.5)	43 (11.1)	5 (50.0)	41 (11.6)	1 (50.0)	6 (8.6)
At least 1 TEAE leading to discontinuation	1 (6.7)	1 (0.2)	1 (9.1)	1 (0.3)	0	0	0	0
At least 1 TEAE leading to death	1 (6.7)	1 (0.2)	1 (9.1)	1 (0.3)	0	0	0	0
Any IRR[d]	6 (40.0)	29	4 (36.4)	19	4 (40.0)	19	1 (50.0)	1
Mild or moderate IRR	6 (40.0)	29 (100.0)	4 (36.4)	19 (100.0)	4 (40.0)	19 (100.0)	1 (50.0)	1 (50.0)

IRR, infusion-related reaction; *SAE*, serious adverse event; *TEAE*, treatment-emergent adverse event.

[a]Treatment duration refers to the total duration in the overall treatment period (studies F01/F02/F03) with reportable data; at the start of study F03 all patients were dose-adjusted to receive 1 mg/kg pegunigalsidase alfa.

[b]Possibly, probably, or definitely related to study treatment.

[c]One severe TEAE of migraine.

[d]IRRs were defined as TEAEs occurring during the infusion or within 2 hours after its completion.

the patient's 25-year smoking history and emphysema and to be unrelated to FD. Only 1 severe TEAE (migraine, reported in 1 patient [6.7%]) was considered as being possibly related to study treatment. Additionally, there was no evidence of transient ischemic attack or stroke during the study.

During the 72-month treatment period, 29 IRRs were reported in 6 patients: 2/8 males (25.0%) and 4/7 females (57.1%). IRRs noted were dizziness and nausea, each reported by 2 patients, and abdominal pain, chest discomfort, chest pain, dyspnea, fatigue, hypotension, infusion reaction, maculopapular rash, paranasal sinus hypersecretion, peripheral swelling, pruritus, and sneezing, each reported by 1 patient. All IRRs were categorized as mild to moderate in severity, with none leading to study withdrawal or death; only 1 IRR (mild peripheral swelling) occurred after the first year of treatment (Table 2).

ADAs

Out of the 15 patients in the study, 4 male patients developed treatment-emergent anti-pegunigalsidase alfa IgG at some point during the study and 11 patients (4 male and 7 female) did not (Figure 2); treatment-emergent ADAs were defined as occurring at a post-baseline visit and not in a patient with ADAs at baseline. Two patients (1 male and 1 female) had anti-pegunigalsidase alfa IgG at baseline before treatment initiation. Of the 5 male patients who were ADA-positive after baseline, 4 were transiently positive and 1 had sustained positivity. Of the 4 transient ADA-positive cases, 1 patient was positive at week 2 but was negative thereafter, and 2 patients had neutralizing antibodies, which were tolerized. The single patient with sustained ADAs tested positive at month 48 and developed neutralizing antibodies at month 54, which persisted until study completion. After the first 6 months of treatment, no more than 2 patients were positive for ADAs at any given time point. At month 60, only 1 patient was positive for ADAs, and neither patient with reported data to 72 months developed ADAs at any point during the study. All ADA-positive samples were further evaluated for their specificity to the polyethylene glycol moieties on pegunigalsidase alfa. Only 1 sample from a male patient who was ADA-positive from visit 2 to visit 9 was positive for anti-PEG antibodies at his visit 5 assessment.

Infusion duration

Infusions were well tolerated, with infusion time decreasing to the minimally allowed duration of 1.5 hours for 7 of 15 patients (47%) in the first year of treatment and for 13 of 13 patients (100%) by month 22. The 2 patients whose infusion times were not decreased to the minimally allowed duration discontinued the study prematurely.

Plasma lyso-Gb3 and Gb3

A reduction from baseline in plasma lyso-Gb3 concentration over time was observed, with the most drastic reductions occurring in the first year of treatment (Figure 3A). After 12 months of pegunigalsidase alfa treatment, the mean (SE; median) plasma lyso-Gb3 concentration was 23.9 ng/mL (5.2; 17.7 ng/mL), a 49.1% reduction from baseline; this reduction continued until 33 months and then stabilized; after 60 months it reached 6.4 ng/mL (1.5; 6.0 ng/mL), an 83.3% reduction from baseline. Baseline values for plasma lyso-Gb3 were 124.4 ng/mL (median: 98.6 ng/mL) in males and 9.6 ng/mL (median: 7.5 ng/mL) in females. Despite these baseline differences, both males and females achieved a mean reduction at 60 months of >70% from baseline (91% reduction for males and 72% reduction for females).

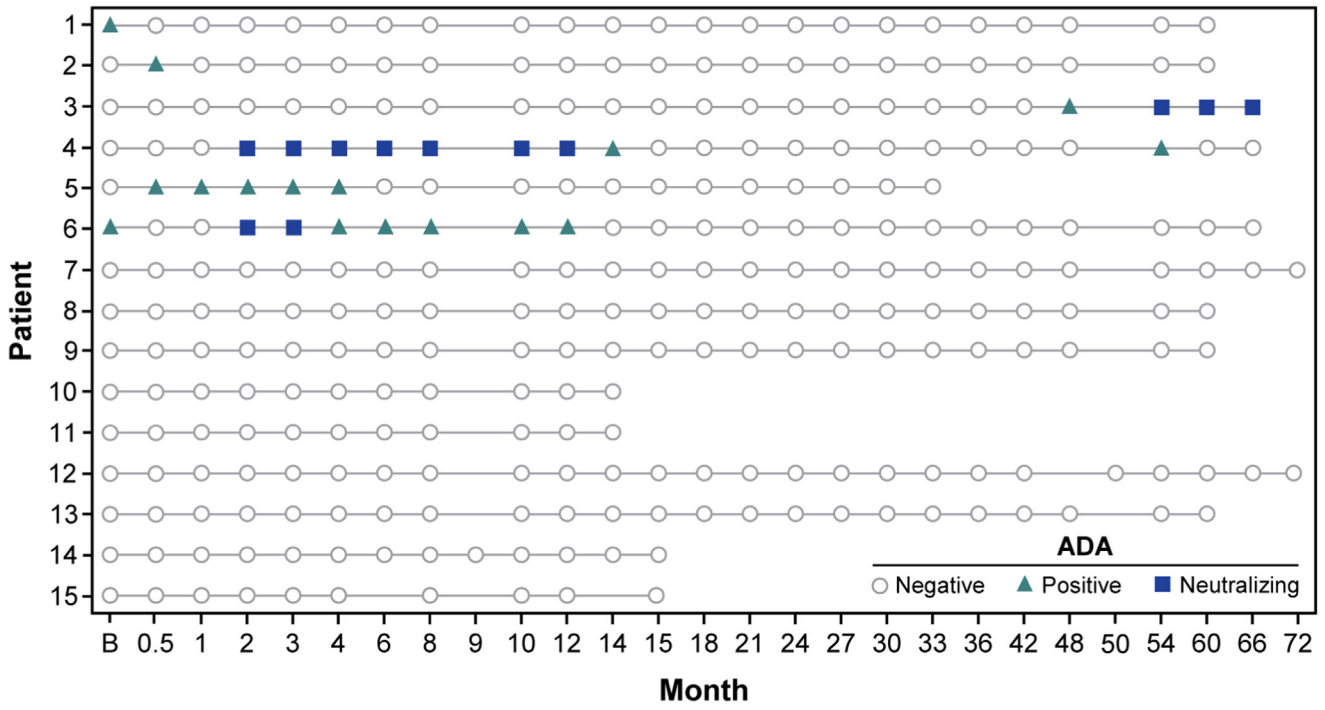

Figure 2 **Antibody status by visit**. Each line represents an individual patient. One patient had a single positive result at week 2; however, the sample was from plasma and not serum as required based on validation. ADA, anti-drug antibody; B, baseline.

Plasma Gb3 concentrations at baseline were 14.86 μg/mL (median: 12.8 μg/mL) for males and 5.99 μg/mL (median: 6.0 μg/mL) for females and, similar to lyso-Gb3, these values decreased most markedly during the first 12 months of treatment (mean [SE; median] decrease of 6.50 μg/mL [1.8; 6.3 μg/mL] for males and 0.33 μg/mL [0.3; 0.3 μg/mL] for females). At 60 months, males had a mean 48% reduction from baseline of 6.72 μg/mL (median: 6.3 μg/mL), whereas females had a mean 41% increase of 2.18 μg/mL (median: 2.7 μg/mL). There was a high correlation (R = 0.963) between the absolute change from baseline to 6 months in mean Gb3 deposition in kidney peritubular capillaries (as was measured as part of F01/F02 studies) and the absolute change from baseline to 24 months in plasma lyso-Gb3 concentrations.

Kidney function

At baseline, UPCR was normal to mildly increased (<150 mg/g) in 10/15 patients (67%) and moderately increased (150-500 mg/g) in 5/15 patients (33%). At month 60, UPCR was normal to mildly increased in 2 of 10 patients (20%) and moderately increased in 8 of 10 patients (80%). No patients developed persistent severe proteinuria during the study. The mean (SE; median) baseline eGFR values were 111.7 (5.5; 114.29) for all patients, 118.1 (7.7; 116.76) for males, and 104.4 (7.5; 105.70) for females (Figure 3B). A slight decrease in eGFR was observed over time and at month 60, mean (SE; median) values were 97.0 (6.4; 101.98) for all patients, 100.0 (8.3; 103.89) for males, and 92.4 (11.4; 93.84) for females; and the calculated mean (SE; median) annualized eGFR slopes up to 72 months were −1.6 (0.8; −1.5) mL/min/1.73 m^2/y for all patients, −2.4 (0.9; −2.8) mL/min/1.73 m^2/y for males, and −0.7 (1.3; −1.3) mL/min/1.73 m^2/y for females.

Cardiac outcomes

Cardiac magnetic resonance imaging results showed stability in LVM, LVMI, and LVEF (Supplemental Table 2). LVM showed an increase at month 60, but all values remained normal.[21] At month 60, mean LVMI (SE) had increased in females by 13.6 g/m^2 (5.3) compared with 5.7 g/m^2 (2.2) in males, and LVEF showed a mean (SE) decrease of 0.5% (1.4) for all patients, 0.6% (1.9) for males, and 0.3% (2.3) for females. Importantly, no left ventricular fibrosis developed over 72 months of treatment. Of the cardiac parameters that were assessed by echocardiography and stress test, most remained stable and within normal ranges (data not shown).

MSSI and patient-reported outcomes

Individual patient MSSI scores showed stability or improvement (data not shown), with a mean (SE) change from baseline in the overall score of −7.5 (1.8) at month 24 and −3.6 (2.3) at month 60 (Supplemental Table 3). All subscores showed decreases (ie, improvements) compared with baseline for most post-baseline visits.

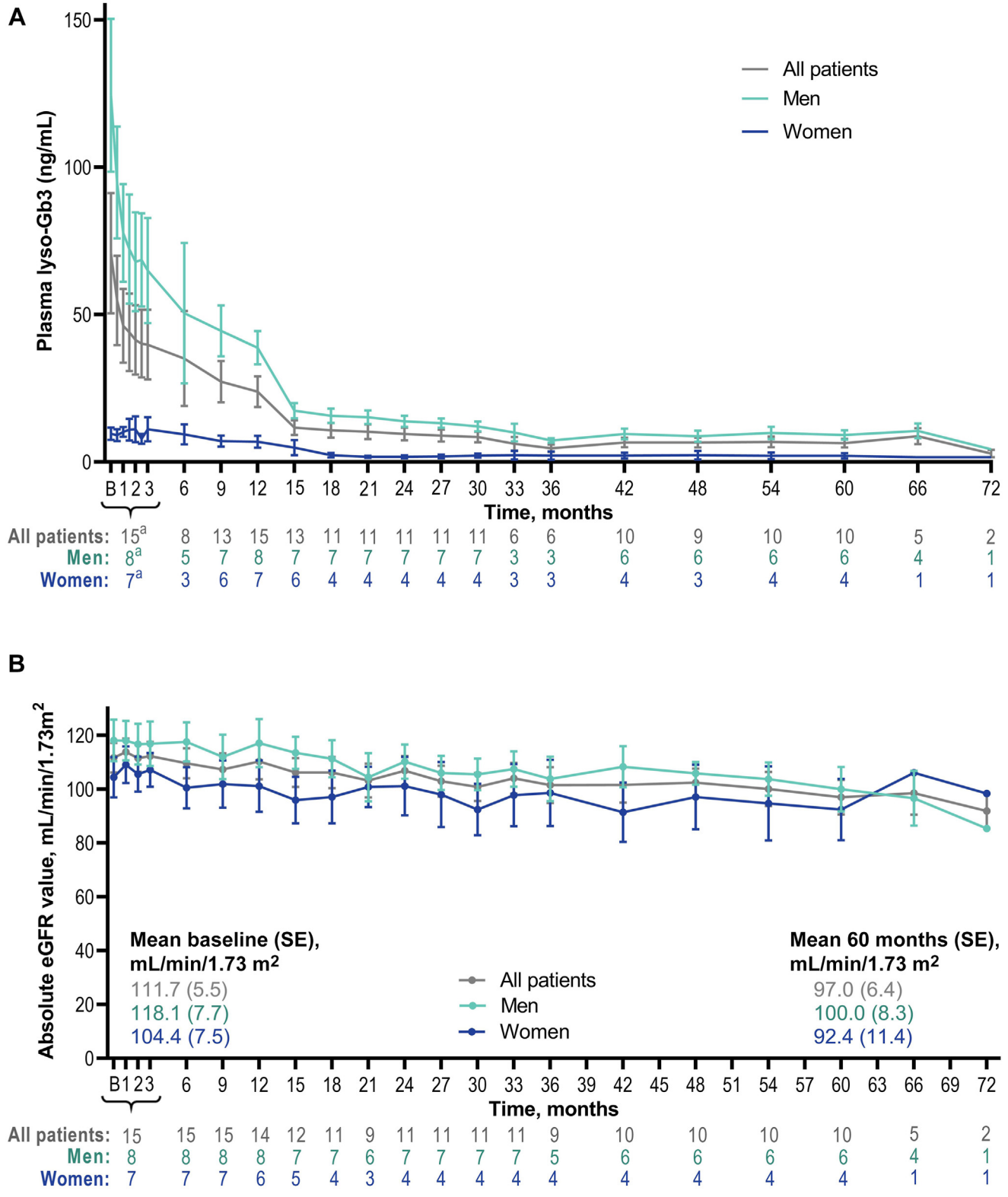

Figure 3 Mean (± SE) (A) plasma lyso-Gb3 over time and (B) absolute eGFR values over time. (A) [a]At month 1.5, $n = 13$ for all patients, $n = 7$ men, and $n = 6$ women. Plasma lyso-Gb3 unaffected reference range: 0.4-1.8 ng/mL; affected Fabry reference range: 2.3-234.9 ng/mL; reference ranges were determined by the UCL Biological Mass Spectrometry Centre from anonymized Fabry disease positive and negative clinical samples. (B) Baseline values are from either visit 1 or screening if visit 1 is not available. B, baseline; eGFR, estimated glomerular filtration rate; lyso-Gb3, globotriaosylsphingosine; SE, standard error; UCL, University College London.

Similarly, pain levels as assessed by BPI-SF average pain score showed stability (change in score = 0) or improvement (change in score <0) for most patients (Supplemental Table 3). After 24 months of treatment, 36.4% of patients (4 of 11; 1 male and 3 female) showed stability in their average pain level compared with baseline, whereas 45.5% of patients (5 of 11; 4 male and 1 female) noted a reduction. At 60 months, these numbers further improved with all patients reporting either stability (30%, 3 of 10; 2 male and 1 female) or improvement (70%, 7 of 10; 4 male and 3 female) in their average pain level, which decreased from an average BPI score of 3.7 out of 10 (0 = no pain, 10 = severe pain) at baseline to 1.8 after 60 months of treatment.

Based on the GSA questionnaire, at baseline most patients reported moderate (40.0%; 3 of 15 male, 3 of 15 female) or severe (13.3%; 1 of 15 male, 1 of 15 female) abdominal pain (Supplemental Table 3). Although no clear trends were observed for the proportions of patients with severity or frequency of abdominal pain or frequency of diarrhea over time, post-baseline evaluations generally showed a reduction in the number of male and female patients with moderate/severe abdominal pain (data not shown).

Discussion

This open-label extension study of a cohort of ERT-naive patients with FD is the first assessment of long-term exposure to pegunigalsidase alfa. We expand the findings of the F01/F02 trials[17] and demonstrate that long-term pegunigalsidase alfa treatment continued to be safe and effective for up to 72 months of treatment. A total of 440 TEAEs occurred during the overall study period, with the majority (86.6%) being unrelated to pegunigalsidase alfa treatment. The occurrence of TEAEs decreased after the first year of treatment, with about half (224 of 440) occurring during the F03 extension study. Of the 59 events that were possibly, probably, or definitely related to study treatment, 58 (98.3%) were mild or moderate in severity; 1 treatment-related event of migraine that occurred during the first year of treatment was severe.

Infusions were well tolerated, with all patients who completed the F03 extension study having their infusion time decreased to the minimally allowed duration of 1.5 hours during the first 2 years of treatment. Further, similar to previously reported data with agalsidase beta, most IRRs occurred during the first year of treatment with only 1 IRR occurring during F03.[22] No IRRs led to treatment discontinuation, and no hypersensitivity reactions occurred after the first year. Treatment-emergent ADAs developed in 4 of 15 (26.7%) patients treated with pegunigalsidase alfa, most during the first year (in F01/F02) and 1 during the fourth year of treatment. This low rate of immunogenicity is similar to what has been reported with other ERTs[22,23] but the transient nature of their expression in most treated patients indicates their prevalence over time may be lower. Neutralizing antibodies were also mostly transient and developed in only 3 of 15 patients (20%), a rate substantially lower than what was reported in previous studies where frequency ranged from 40%-77% in adult patients treated with agalsidase alfa or agalsidase beta.[10,11] Importantly, studies have demonstrated that formation of neutralizing ADAs is associated with reduced overall therapeutic effectiveness.[11-13]

Males have higher mean plasma lyso-Gb3 and Gb3 concentrations at baseline and greater mean reductions from baseline during treatment compared with females.[24,25] However, in our trial, plasma lyso-Gb3 concentrations in both sexes steadily decreased from baseline and remained lower than baseline throughout the follow-up period of up to 72 months of treatment, with mean values at 60 months of 9.2 ng/mL for males and 2.1 ng/mL for females. Plasma Gb3 concentrations also decreased at 12 months in males and females by a mean (SE) of 6.50 μg/mL (1.8) and 0.33 μg/mL (0.3), respectively, and remained relatively stable up to 60 months.

Treatment with pegunigalsidase alfa was also associated with reduction of eGFR decline comparable to that seen with other ERTs[26] and stable outcomes in other organ systems where complications are often observed in patients with FD.[27] At baseline, most patients (67%) had normal to mildly increased UPCR, whereas at month 60, 20% of patients had mild to moderately increased UPCR and 80% had moderately increased UPCR. The number of patients who had moderately increased UPCR over the course of the study can be expected in patients with progressive kidney disease, which is often not completely manageable by ERT alone in adult patients with FD.[28] Similar to what is seen with other ERTs,[26] overall decline in kidney function was reduced, with an overall mean (SE) annualized eGFR slope of −1.6 (0.8) mL/min/1.73 m^2/y (−2.4 [0.9] mL/min/1.73 m^2/y for males and −0.7 [1.3] mL/min/1.73 m^2/y for females). Additionally, most patients progressed from mild to moderate proteinuria to moderate proteinuria. The impact of potential presence or absence of concomitant medications, such as renin-angiotensin-aldosterone system blockers, on this specific end point was not evaluated. Importantly, eGFR slope, calculated using a linear regression model normalized by the time point of observation, takes into account all time points, whereas absolute change considers just 2.[19] Therefore, eGFR slope is more reliable as a predictive instrument over time because the higher number of time points dilutes the variability of individual eGFR values, which may be subject to perturbation and improperly reflect renal function of the patient.

At 60 months, LVEF showed a marginal mean (SE) decrease of 0.5% (1.4). Throughout the study LVM, LVMI, and LVEF values remained within normal ranges and were considered normal or stable. Cardiac characteristics of mean (SE) LVMI and LVEF at baseline were 52.7 g/m^2 (3.7) and 59.9% (2.2), respectively. LVMI maximum baseline values of 73 g/m^2 for males and 54 g/m^2 for females indicated that none of the patients showed cardiac hypertrophy because

they were below the thresholds of 91 g/m^2 for males and 77 g/m^2 for females.[21] Importantly, there was no evidence of transient ischemic attack or stroke and no patients developed cardiac fibrosis during the study.

MSSI and patient-reported outcome measures showed improvement or stability in most patients. Improvement was seen in the overall change in MSSI at 24 and 60 months. The renal MSSI sub-score showed improvement at all time points despite an increased number of patients with moderately increased UPCR and negative eGFR slope. This may be explained by the broader categories in the MSSI, which may not capture subtle changes that can be observed with the more precise UPCR and eGFR measurements. Improvement was also seen in the BPI-SF scores of 70% of patients at 60 months, with the remaining 30% of patients reporting stability via their BPI-SF scores. Improvement or stability in pain, which has a major impact on the quality of life in patients with FD, was seen in most male and female patients who completed the study for both the BPI-SF questionnaire and the GSA questionnaire.

This study was limited by the small number of patients, especially at the 72-month time point ($n = 2$), and lack of a control group. Additionally, we cannot fully understand the potential impact of concomitant medication administration (eg, renin-angiotensin-aldosterone system blockers) on end points evaluating kidney function, such as UPCR, over the study duration.

Conclusions

This is the first assessment of long-term administration of pegunigalsidase alfa in patients with FD. The results presented here show that the favorable safety and immunogenicity profile of long-term pegunigalsidase alfa treatment was consistent with previous studies,[17] indicated by the low rate of TEAEs, similar or lower immunogenicity than other currently available ERTs, and the transient nature of the observed ADAs. Additionally, long-term treatment with pegunigalsidase alfa has continued to provide clinical benefit, indicated by moderate decline in renal end points, comparable to other ERTs,[26] and stability in cardiac end points over the 6-year study period.

A plain language summary of this study is available as supplemental material (Supplemental Text: Long-term safety and efficacy of pegunigalsidase alfa in adult patients with Fabry disease).

Data Availability

At this time, we will approve or deny data requests from external parties on a case-by-case basis. Chiesi reserves the right to deny requests for any and all legally appropriate reasons. Data requests that risk sharing participant-level data or proprietary information will not be approved.

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Ethics Declaration

The study protocol and any amendments were reviewed by an Institutional Review Board or Independent Ethics Committee. The main Institutional Review Board was the Research Ethics Committee in Asuncion, Paraguay. The Investigator obtained written consent from patients before study enrollment. The study was conducted in accordance with the ethical principles expressed in the Declaration of Helsinki, the approved protocol, Good Clinical Practice guidelines, and applicable regulatory requirements.

Conflict of Interest

Derralynn Hughes has received speaker's honoraria from Amicus Therapeutics, Takeda, Sanofi, Freeline, Protalix, and Idorsia. Derlis Gonzalez has been or is currently involved in clinical trials with Takeda, Protalix, and mAbxience. Gustavo Maegawa has received grants, personal and consultant fees from Sanofi; grants from Pfizer Inc.; personal fees from Sio Gene Therapies; grants from NIH/NINDS, and personal fees from NIH/CSR. John A. Bernat receives research support from Avrobio, BioMarin Pharmaceutical, Chiesi Farmaceutici, Idorsia Pharmaceuticals, Pfizer, Protalix Biotherapeutics, Sangamo Therapeutics, Sanofi, Takeda, and Travere Therapeutics; has received a speaker honorarium from the Fabry Support and Information Group; and has

participated in advisory boards for Chiesi USA, Sanofi, and Takeda. Myrl Holida has received speaker honoraria from Protalix and Chiesi and has participated in advisory boards for Amicus and Sanofi. Pilar Giraldo has been involved in pre-marketing studies with Genzyme, Protalix, and Idorsia and has received grants from Sanofi and Takeda; monies received for these activities have been deposited into the Spanish Foundation for the Study and Treatment of Gaucher Disease (FEETEG) to contribute to the development of research in lysosomal storage disorders. Mohamed G. Atta declares no conflicts of interest. Sari Alon is a full-time employee of Protalix. Raul Chertkoff and Einat Brill Almon were full-time employees of Protalix at the time of study conduct and are now consultants to Protalix. Rossana Rocco is a full-time employee of Chiesi. Ozlem Goker-Alpan has conducted contracted research with Amicus, Freeline, Genentech, Protalix, Sangamo, Sanofi, Takeda, 4DMT, Avrobio; received consulting fees from Amicus, Sanofi, Takeda, Sangamo, 4DMT, Avrobio; served on advisory boards for Amicus, Sanofi, Takeda, Sangamo, 4DMT, Avrobio; and participated in speakers' bureaus for Sanofi, Takeda.

Additional Information

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