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ORIGINAL ARTICLE

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Pegunigalsidase alfa, a novel PEGylated enzyme replacement therapy for Fabry disease, provides sustained plasma concentrations and favorable pharmacodynamics: A 1-year Phase 1/2 clinical trial

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Abstract

Pegunigalsidase alfa, a novel PEGylated, covalently crosslinked form of α-galactosidase A developed as enzyme replacement therapy (ERT) for Fabry disease (FD), was designed to increase plasma half-life and reduce immunogenicity, thereby enhancing efficacy compared with available products. Symptomatic adults with FD participated in this open-label, 3-month dose-ranging study, followed by a 9-month extension. Three cohorts were enrolled in a stepwise manner, each receiving

increased doses of pegunigalsidase alfa: 0.2, 1.0, 2.0 mg/kg, via intravenous infusion every other week. Pharmacokinetic analysis occurred on Day 1 and Months 3, 6, and 12. Kidney biopsies at baseline and Month 6 assessed peritubular capillary globotriaosylceramide (Gb3) content. Renal function, cardiac parameters, and other clinical endpoints were assessed throughout. Treatment-emergent adverse events (AEs) and presence of immunoglobulin G (IgG) antidrug antibodies (ADAs) were assessed. Sixteen patients completed 1 year's treatment. Mean terminal plasma half-life (each cohort) ranged from 53 to 121 hours. All 11 male and 1 of 7 female patients presented with classic FD phenotype, in whom renal peritubular capillary Gb3 inclusions were reduced by 84%. Mean estimated glomerular filtration rate was 111 mL/min/1.73 m^2 at baseline, remaining stable throughout treatment. Three patients developed treatment-induced IgG ADAs; following 1 year's treatment, all became ADA-negative. Nearly all treatment-emergent AEs were mild or moderate. One patient withdrew from the study following a serious related AE. Pegunigalsidase alfa may represent an advance in ERT for FD, based on its unique pharmacokinetics and apparent low immunogenicity.

KEYWORDS

antidrug antibodies, enzyme replacement therapy, Fabry disease, immunogenicity, pegunigalsidase alfa, pharmacokinetics

1 | INTRODUCTION

Fabry disease (FD) (OMIM #301500) is an X-linked genetic disorder caused by a deficiency in the activity of the lysosomal enzyme alpha-galactosidase A (α-Gal A) (EC 3.2.1.22), resulting from more than 900 mutations in the *GLA* gene.[1,2] Classically affected male patients typically have no or very low residual enzyme activity and demonstrate accumulation of globotriaosylceramide (Gb3) within tissues and organs, where it is thought to contribute to serious progressive pathology in the kidneys, cardiovascular system, and nervous system.[2] Patients with residual enzyme activity, including heterozygote females, may also develop a range of similar symptoms, typically with a later onset and a variable severity.[3] Some female patients may remain asymptomatic throughout life.

Two enzyme replacement therapies (ERTs) for FD have been commercially available since 2003: agalsidase beta and agalsidase alfa.[4,5] The pharmacokinetic (PK) profiles of the two drugs are similar, with half-lives of approximately 2 hours.[4,5] In clinical studies of these drugs, treated patients have demonstrated a reduction in Gb3 accumulation, improvement in neuropathic pain, and trends toward improvement in renal function and reduction in major clinical events.[6–9]

Pegunigalsidase alfa is a novel, PEGylated, chemically modified α-Gal A enzyme, developed as an ERT for the treatment of FD. The enzyme is produced in a plant cell-based ProCellEx system.[10] Chemical modification with a homo-bifunctional polyethylene glycol (PEG, 2000 Da) results in covalently crosslinked monomers with preserved 3D structure.[11] As part of the chemical modification, additional PEG moieties are attached to surface lysine residues by one end only. In *in vitro* testing, pegunigalsidase alfa showed greater stability than agalsidase alfa and agalsidase beta in human plasma at 37°C and under simulated lysosomal-like conditions.[11] In Fabry mice, compared with agalsidase alfa, pegunigalsidase alfa demonstrated prolonged plasma half-life and an enhanced biodistribution, including increased enzymatic activity in the heart and kidney and reduced clearance by the liver.[11]

This article presents the results of the 1-year treatment experience in two Phase 1/2 clinical studies. The first study (NCT01678898) was a 3-month dose-ranging study followed by a 9-month extension study (NCT01769001). These studies were designed to evaluate the pharmacokinetics of pegunigalsidase alfa and assess the efficacy, safety, and tolerability of the drug when administered to treatment-naïve FD patients.

2 | METHODS

2.1 | Study ethics

Both study protocols were reviewed and approved by an institutional review board or ethics committee prior to initiation. All patients provided informed written consent to participate before study enrollment. Studies were conducted in

accordance with the ethical principles expressed in the Declaration of Helsinki, approved protocol, Good Clinical Practice guidelines, and applicable regulatory requirements.

2.2 | Patients

FD symptomatic adults ≥18 years old were eligible for the dose-ranging study. FD was confirmed in male patients by plasma and/or leucocyte α-Gal A activity below the lower limit of normal (plasma, <3.2 nmol/h/mL; leucocytes, <32 nmol/h/mg protein). In females, FD was confirmed by identification of a pathogenic variant in one of the FD *GLA* genes. Additional inclusion criteria included being naive to ERT or not having received ERT in the previous 6 months and negative for anti-pegunigalsidase alfa IgG antibodies, and estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2. Key exclusion criteria included chronic kidney disease stages 3 to 5 or history of renal dialysis or transplantation, angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) therapy dose change or initiation within 4 weeks before screening, severe myocardial fibrosis defined as ≥2 late-enhancement positive ventricular segments, or a history of stroke.

2.3 | Study design

The study was an open-label, multinational, multicenter, 3-month dose-ranging study of pegunigalsidase alfa for treatment of FD. Three cohorts of patients were enrolled in a stepwise manner and treated with one of three increasing dose levels of pegunigalsidase alfa: 0.2 mg/kg, 1.0 mg/kg, or 2.0 mg/kg via intravenous infusion every other week (EOW) (details presented in Supporting Information Appendix S1). Patients completing the 3-month study were invited to participate in the extension study, continuing to receive their original dose for 9 additional months.

2.4 | Pegunigalsidase alfa dosing

Infusions of pegunigalsidase alfa were administered every 2 weeks (±3 days) at approved medical centers, and patients observed for 24 hours after each of the first seven infusions (3 months of treatment). For the 0.2- and 1.0-mg/kg cohorts, each dose was diluted with 0.9% NaCl to a volume of 150 mL, and in the 2.0-mg/kg group to a final volume of 350 mL. The mean durations of the initial infusions in the 0.2-mg/kg, 1.0-mg/kg, and 2.0-mg/kg groups were 4.0, 5.5, and 6.4 hours, and were sequentially reduced per protocol to mean durations of 1.5, 3.3, and 3.1 hours at 12 months, respectively. In the 2.0-mg/kg group, premedication consisting of standard doses of H1 and H2 histamine receptor blockers was administered at 12 hours and 2 hours prior to the start of each infusion and was sequentially reduced and/or discontinued as tolerated.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study. Proof that informed consent was obtained is available upon request. This article does not contain any studies with animal subjects performed by any of the authors.

3 | ASSESSMENTS

3.1 | Pharmacokinetics

Venous blood samples for pharmacokinetic analysis were collected into EDTA tubes on Day 1, and at 3, 6, and 12 months at the following time points: pre-infusion; 1 hour after infusion start; at the end of the infusion; at 1, 4, 8, 24, 48 ± 2, 72 ± 3, and 96 ± 3 hours; and at 2 weeks ±3 days after the end of the infusion. Pegunigalsidase alfa concentrations were determined using a validated enzyme-linked immunosorbent assay (ELISA) with a lower limit of quantitation of 20 ng/mL in plasma. The following noncompartmental pharmacokinetic parameters were derived from the plasma concentration-time curves using Phoenix WinNonlin 6.3 (Certara, L.P., Princeton, New Jersey): maximum observed concentration (C_{max}), terminal half-life ($T_{1/2\gamma}$), area under the plasma concentration-time curve from 0 hours to infinity ($AUC_{0-\infty}$), volume of distribution during the elimination phase (Vz), and plasma clearance (Cl).

3.2 | Pharmacodynamics

Kidney biopsies obtained at baseline and after 6 months of treatment were fixed in glutaraldehyde and embedded in resin. Semithin sections were obtained and stained with toluidine blue, and glass slides were scanned into whole slide images (at 100x). Three hundred cortical peritubular capillaries per case were digitally annotated and blindly assessed by three renal pathologists for Gb3 inclusion using the quantitative Barisoni Lipid Inclusion Scoring System (BLISS).[12]

Plasma Gb3 and lyso-Gb3 concentrations were assayed in venous blood obtained before dosing initiation, at each study visit in the first 3 months, and every 3 months thereafter. Plasma lyso-Gb3 was extracted and quantified according to Boutin and colleagues,[13] with minor modifications: dimethyl psychosine was used as an internal standard in place of *N*-glycinated lyso-ceramide. Plasma Gb3 was extracted and quantified according to Mills and colleagues.[14]

3.3 | Clinical effect

The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation.[15] Proteinuria was determined from a spot urine sample and expressed as the ratio of urine protein to creatinine excretion (UPCR; mg/g). These renal variables were assessed at every visit during the first 3 months and every 3 months thereafter. Cardiac MRI was performed at baseline and after 6 and 12 months of treatment to estimate left ventricular mass (LVM), LVM index (LVMi), and ejection fraction (EF), and to assess the presence of cardiac fibrosis. Pain was assessed using the Brief Pain Inventory (BPI; Short Form) at baseline and after every 3 months of treatment. The Mainz Severity Score Index (MSSI) was used to assess the overall burden of FD symptoms at baseline and after every 6 months of treatment.[16] Effect of treatment on severity and frequency of abdominal pain and frequency of diarrhea was assessed using a questionnaire described by Dehout et al[17] given to patients at baseline and every 3 months thereafter.

3.4 | Safety

Treatment-emergent adverse events (TEAEs), clinical laboratory measurements, physical examinations, and electrocardiographic findings were assessed during the study. The presence and titers of anti-pegunigalsidase alfa immunoglobulin G (IgG) antibodies were determined by a validated direct ELISA. Antidrug antibody (ADA)-positive samples were further characterized, including their neutralizing activity, using an enzymatic activity assay. These assays were developed and validated based on the current immunogenicity guidelines from the United States Food and Drug Administration and European Medicines Agency.

3.5 | Analysis

Because of the small number of patients at each dose level and the study design, only descriptive statistics are presented as mean ± SD unless otherwise stated. The results are summarized for the following groups of patients: all patients and patients with a classic phenotype presentation of FD. Classic phenotype FD was defined as patients with low (<30% of the normal laboratory mean) values of α-Gal A activity and at least one FD-specific symptom, such as neuropathic pain, cornea verticillata, or clustered angiokeratoma.

4 | RESULTS

4.1 | Patients

Forty-two patients from 13 sites were screened for eligibility, with 19 patients from 11 sites enrolled. Of the 23 screening failures, 9 had α-Gal A activity inconsistent with FD and 14 failed to meet other inclusion criteria. One patient designated for the 1.0-mg/kg group voluntarily withdrew from the study before receiving study treatment. Thus, the safety population comprised 18 patients receiving any dose of study drug. Two male patients in the 1.0-mg/kg treatment group discontinued the study: one experienced a hypersensitivity reaction (bronchospasm) during the first infusion, and one was noncompliant. All 11 male patients and a single female patient were classified as having classic phenotype FD. Of the 16 treated patients who completed the study, four were treated with an ACE inhibitor or ARB at baseline. Of the 16 patients who completed 12 months of treatment, 15 enrolled in an extension study (NCT01981720). Baseline characteristics are presented in Table 1 (patient disposition and summary baseline characteristics of the safety population are presented in Supporting Information Appendix S1, figure 1 and table 1, respectively).

4.2 | Pharmacokinetics

Measurable concentrations of pegunigalsidase alfa were present throughout the entire 14-day dosing interval (Figure 1). Calculated pharmacokinetic parameters (Supporting Information Appendix S1, table 2) C_{max} and $AUC_{0-\infty}$ demonstrated dose-related increases on each sampling day. $T_{1/2\gamma}$, Cl, and Vz did not systematically change with increasing dose within each sampling day but did exhibit variability among the three doses. The mean $AUC_{0-\infty}$ increased between Day 1 and 3 months for all dose levels. Subsequent increases in $AUC_{0-\infty}$ with increasing treatment duration were observed for the 1.0- and 2.0-mg/kg dose levels, but not at 0.2 mg/kg. Overall mean $T_{1/2\gamma}$ was about 80 hours and ranged from 53 to 121 hours.

4.3 | Pharmacodynamics

Kidney biopsies from three patients were excluded from this analysis: one from a male not handled according to protocol, one from a female with no cortical tissue, and one from a male patient that revealed minimal tissue involvement (baseline BLISS score of 0.44). In the remaining 13 patients analyzed, a mean decrease in peritubular capillary Gb3 content of 67.8% ± 8.9% was observed after 6 months, and in the eight patients with classic phenotype FD who were analyzed, a mean decrease of 84.1% ± 3.3% was found (Figure 2). At the 6-month time point, 11 of the 13 patients demonstrated more than 50% reduction in the number of Gb3 inclusions.

Reductions in plasma Gb3 and lyso-Gb3 levels were observed throughout the study in the entire population

TABLE 1 Patient baseline characteristics

	Dose group																	
Pegunigalsidase alfa dose group	**0.2 mg/kg**						**1 mg/kg**								**2 mg/kg**			
Patient	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19
Gender	F	F	M	M	M	M	F	M	M	M	M	M	M	F	F	F	F	M
Age (y)	22	34	27	51	26	23	33	28	28	52	28	36	18	50	21	54	54	34
Race[a]	W	W	B/AA	O, ME	W	W	W	W	W	W	W	W	B/AA	B/AA	W	W	W	W
Ethnicity (Hispanic/Latino)[b]	Y				Y													
α-Gal A activity in leucocytes[c] (% normal)	15.0 (18%)	40.0 (48%)	1.6 (2%)	2.0 (2%)	4.0 (5%)	5.0 (6%)	72.0 (86%)	2.4 (3%)	3 (4%)	2.2 (3%)	7.8 (9%)	0.61 (1%)	0.0 (0%)	67.0 (80%)	33.0 (40%)	42.0 (50%)	53.0 (63%)	0.56 (1%)
α-Gal A activity in plasma[d] (% normal)	2.0 (15%)	4.3 (35%)	0.1 (1%)	0.15 (1%)	0.0 (0%)	0.4 (3%)	5.8 (45%)	0.17 (1%)	0.4 (3%)	0.23 (2%)	0.44 (3%)	0.05 (0%)	0.4 (3%)	7.8 (60%)	4.1 (32%)	7.8 (60%)	2.52 (19%)	0.4 (3%)
Plasma lyso-Gb3 (ng/mL)	19.2	7.5	66.5	84.7	272.9	112.5	14.40	123.0	87.1	29.9	5.1	193.4	80.8	6.80	3.40	4.95	10.8	61.80
Characteristic facies[b]				Y				Y										Y
Angiokeratomas[b]		Y		Y		Y	Y	Y	Y	Y		Y				Y		Y
Cornea verticalata[b]	Y	Y		Y	Y	Y	Y	Y				Y			Y	Y	Y	Y
Diaphoresis[b] (hyper/hypo)	Y	Y		Y		Y	Y		Y	Y	Y	Y	Y		Y	Y	Y	Y
Abdominal pain[b]	Y		Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y		Y	Y
Diarrhea/Constipation[b]			Y	Y	Y		Y	Y	Y		Y	Y	Y		Y		Y	Y
Acroparesthesias[b]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y		Y	Y
Genotype	c.572 T > C	c.802-2A > T	c.806 T > A	c.679C > T	c.646dup	c.894 T > A	c.402 T > G	c.444 T > G	c.1042 dup	c.98A > G	c.644A > G	c.401A > C	c.400 T > G	c.400 T > G	c.803_806 del	c.803_806 del	c.548G > T	c.1025G > A
Mutation type[e]	M		M		Ta		N	M	N	M	M	M	M	M	T	T	M	M
Phenotype[f]	C		C	C	C	C		C	C	C	C	C	C					C

[a]AA, African American; B, Black; ME, Middle Eastern; O, other; W, White.
[b]Y, yes.
[c]Units: nmol/h/mg protein; normal range: 33 to 134.
[d]Units: nmol/h/mL; normal range: 4 to 21.9.
[e]M, missense; N, nonsense; T, truncated protein; Ta, truncated after 230aa.
[f]C, classic.

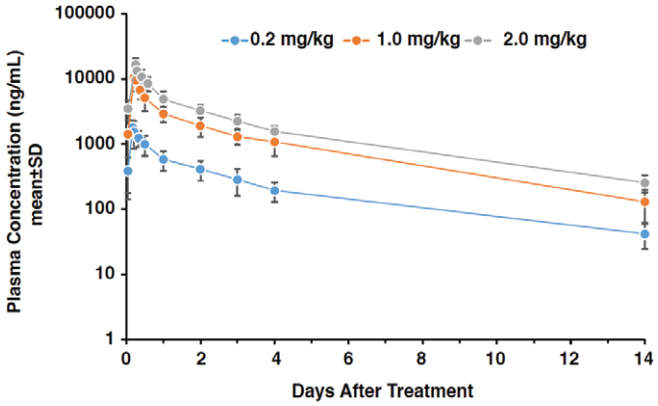

FIGURE 1 Pegunigalsidase alfa plasma levels following dosing on Day 1. SD, standard deviation

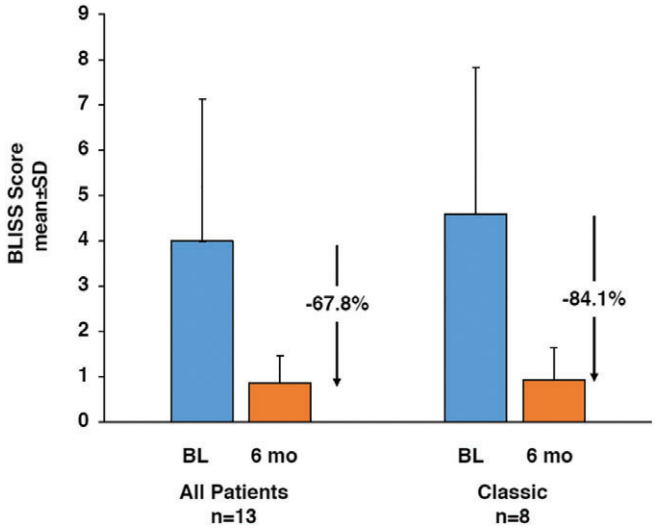

FIGURE 2 Reduction of Gb3 deposition in kidney peritubular capillaries following 6 months of pegunigalsidase alfa treatment. BL, baseline; BLISS, Barisoni Lipid Inclusion Scoring System; Gb3, globotriaosylceramide; SD, standard deviation. The three dosing groups were combined for this analysis

including patients with classic phenotype FD (details presented in Supporting Information Appendix S1, Results; figure 2).

4.4 | Clinical effect

At baseline, mean eGFR was 111.2 ± 20.9 mL/min/1.73 m^2 (range: 78-156 mL/min/1.73 m^2). In the entire study population, minimal change in mean eGFR of −0.8 ± 7.7 mL/min/1.73 m^2 was observed over the 12 months of treatment (12-month mean: 110.5 ± 23.4 mL/min/1.73 m^2; range: 68-152 mL/min/1.73 m^2). In the 10 patients with classic phenotype FD, the mean annualized eGFR slope (calculated from monthly measurement, total of 6-7 data points per patient) was −1.8 mL/min/1.73 m^2/year (range: −18.18-6.35 mL/min/1.73 m^2/year). Upon excluding one male patient who was treated with doxycycline, which may transiently exacerbate renal disease,[18] the remaining group of nine classic patients demonstrated a mean eGFR slope of 0.01 mL/min/1.73 m^2/year (range: −6.35-6.35 mL/min/1.73 m^2/year) (Supporting Information Appendix S1, figure 3).

At baseline, 12 patients had normal UPCR and four had UPCR above normal (<200 mg/g). After 12 months, 14 patients had normal UPCR levels (baseline and final UPCR measurements are presented in Supporting Information Appendix S1, table 3). Two additional patients with the highest baseline UPCR demonstrated a decrease during treatment (a female patient improved from 405 to 240 mg/g, and a male patient improved from 298 mg/g to 209 mg/g). No patient initiated or increased the dose of an ACE inhibitor or ARB during treatment.

Other exploratory clinical outcomes are presented in detail in the Supporting Information Appendix S1. Briefly, at baseline, mean LVM and LVMi were within the normal range, and no cardiac fibrosis was present. Cardiac MRI results showed that the majority of patients maintained cardiac parameters (LVM, LVMi, and EF) within the normal ranges throughout the 12-month study, with a slight decrease in LVMi in patients with classic phenotype FD. No new fibrosis was observed. Improvements in BPI pain scores and in each subscale of the MSSI were observed during the study. A favorable trend was seen in gastrointestinal symptoms over 12 months.

4.5 | Safety

A total of 223 TEAEs were reported in 17 of the 18 treated patients, and 169 (75.8%) were deemed to be not related or unlikely to be related to treatment. All TEAEs were of mild or moderate intensity, with the exception of four patients who each experienced a TEAE that was considered severe, including pain in extremity, renal hematoma due to kidney biopsy at baseline, migraine, and bronchospasm. Of these four events, migraine and bronchospasm were considered possibly related and definitely related to treatment, respectively. The bronchospasm event occurred 40 minutes after starting the first infusion of pegunigalsidase alfa, was treated with epinephrine and corticosteroids, and resolved without sequelae. The patient was withdrawn from the study per protocol, and this event was reported as a related serious adverse event (AE). This patient had pre-existing IgE anti-pegunigalsidase antibodies.

The most common TEAEs in the safety population were fatigue (n = 6 patients; 33%), nausea (n = 5; 28%), and vomiting (n = 5; 28%). The most common TEAEs considered possibly related to treatment were nausea (n = 4; 25%) and chest discomfort, dizziness, maculopapular rash, and fatigue (n = 2 patients each; 13%). Eight patients (including

TABLE 2 Adverse events occurring during or within 2 hours of completion of an infusion of pegunigalsidase alfa

Dose (mg/kg)	Subject	Visit	Adverse events (CRF term/MedDRA preferred term)	Treatment relationship
0.2	1	1	Headaches/Headache	Unrelated
	3	3	Generalized itching/Pruritus generalized	Unlikely
	2	1, 3	Chest tightening/Chest discomfort	Possibly
		1	Sneezing/Sneezing	Possibly
		3	Nausea/Nausea	Possibly
		6	Sweating/Hyperhidrosis	Unlikely
		27	Sneezing and sinus drainage/Paranasal sinus hypersecretion	Possibly
1.0	7	2, 15	Hypotension/Hypotension	Probably
		3	Lightheadedness/Dizziness	Possibly
		9	Shortness of breath/Dyspnea	Possibly
		13	Maculo-papular erythematous/Rash maculo-papular	Probably
	8	6, 8	Infusion reaction/Infusion related reaction	Possibly
		15	Pain at left chest/Chest pain	Possibly
		17	Itching/Pruritus	Possibly
		17	Rash/Rash	Unlikely
		18	Rash at tape site/Dermatitis contact	Unrelated
		20	Nausea/Nausea	Possibly
		20	Dizziness/Dizziness	Possibly
	11	1	Bronchospasm/Bronchospasm	Definitely
2.0	17	1-7	Abdominal cramping/Abdominal pain	Possibly
	18	3	Epigastric pain/Abdominal pain upper	Unrelated

Abbreviations: CRF, case report form; MedDRA, Medical Dictionary for Regulatory Activities.

the patient with bronchospasm) experienced a total of 30 TEAEs that occurred during or within 2 hours after infusion of pegunigalsidase alfa (Table 2); 24 of these TEAEs were deemed to be probably, possibly, or definitely related to treatment. The majority of laboratory hematology, biochemistry, and urinalysis parameters remained within normal levels, and no clinically important mean changes from baseline were observed.

4.5.1 | Antibodies

Three patients, all male, developed treatment-induced IgG ADAs following treatment with pegunigalsidase alfa: two patients in the 0.2-mg/kg group (maximum titers 2198 and 4633) and one in the 1.0-mg/kg group (maximum titer 237). No patient in the 2.0-mg/kg group developed treatment-induced ADAs. All three patients became ADA-negative after approximately 1 year of treatment, suggesting immune tolerance induction. The ADAs in the two patients in the 0.2-mg/kg group were transiently neutralizing but became non-neutralizing as treatment continued, with transient and reversible effects on pharmacokinetics (i.e., an increase in enzyme clearance and a decrease in $T_{1/2\gamma}$; Figure 3). No influence of ADAs on pharmacodynamics, efficacy, or safety responses was evident.

5 | DISCUSSION

ERT for the treatment of FD has been available since the early 2000s. However, beneficial clinical responses have not been consistently reported and have not been as robust as initially anticipated.[2,19–21] Here, we report that treatment with pegunigalsidase alfa in FD patients results in an extended drug plasma coverage and low immunogenicity that distinguish this novel ERT from the two commercially available products. Furthermore, we report the specific effects of the presence of ADAs on drug PK, which, to our knowledge, is the first report of its kind.

Compared with previous pharmacokinetic reports for agalsidase alfa and beta, both of which exhibit a $T_{1/2\gamma}$ of $\leq$2 hours and maintain measurable plasma levels for <24 hours,[4,5] pegunigalsidase alfa had a $T_{1/2\gamma}$ of about 80 hours, with measurable plasma levels sustained for the entire 2-week dosing interval, thus providing an active enzyme reservoir in the circulation to reach the target tissues. The covalent crosslinking, keeping the protein in its

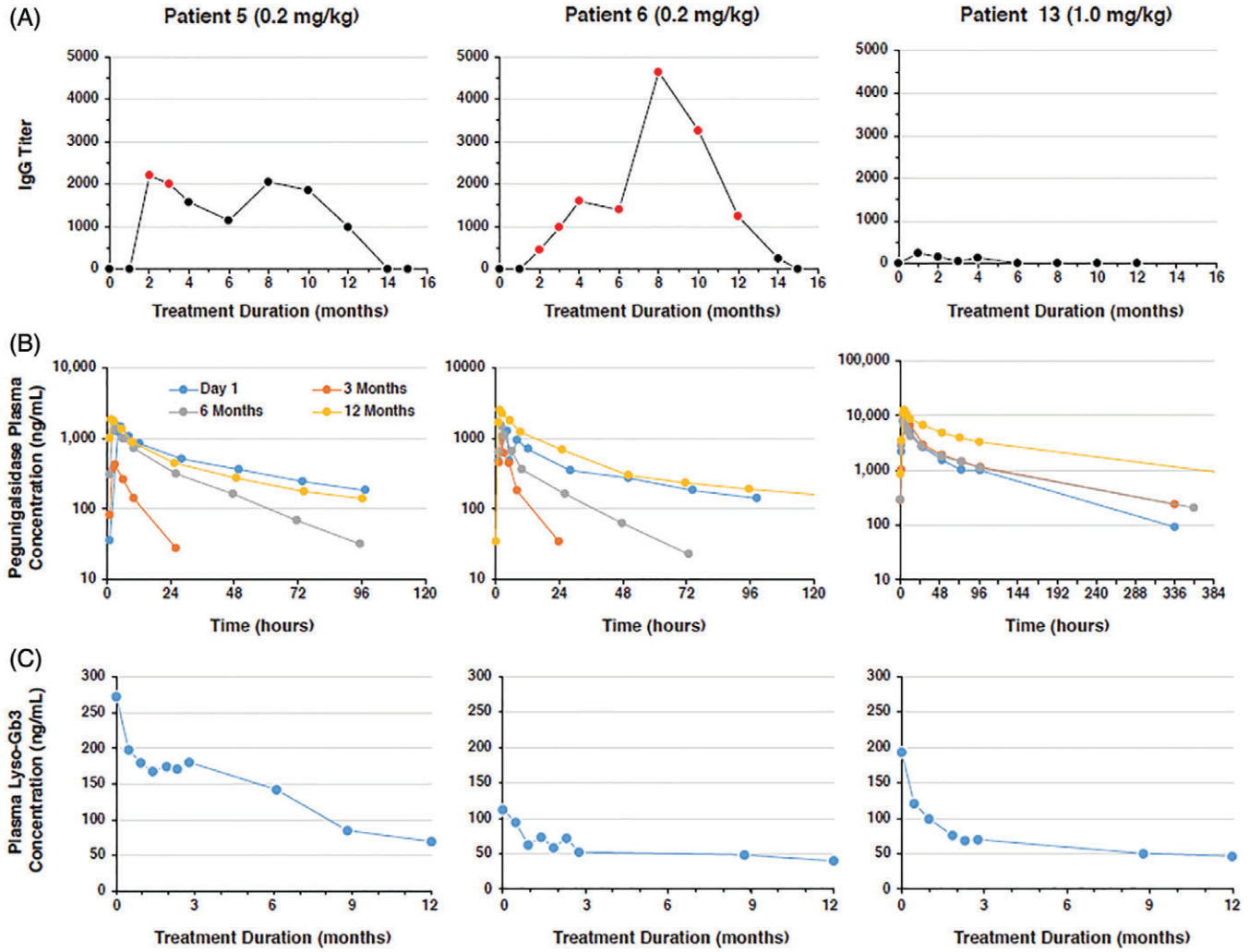

FIGURE 3 The effect of ADA positivity on pharmacokinetics and pharmacodynamics of pegunigalsidase alfa. (A) IgG ADA titer, with red symbols indicating neutralizing antibodies. (B) Pharmacokinetic profiles on Day 1 and at Months 3, 6, and 12. (C) Lyso-Gb3 levels. ADA, antidrug antibody; IgG, immunoglobulin G; lyso-Gb3, lyso-globotriaosylceramide. The IgG titers in these three patients have now been followed for at least 24 months and have remained at 0

homodimer-active configuration and maintaining stable enzymatic activity, combined with additional PEG moieties attached to the protein surface, contribute to the prolonged plasma half-life and subsequent delivery to target tissues[11] and may mask epitopes responsible for generating an immune response.[22] Preliminary *in vitro* experiments demonstrated less recognition of pegunigalsidase alfa compared to agalsidase alfa or beta.[23] Immunogenicity is an important concept in the use of ERT in FD. In clinical trials, 68% and 73%[6,24] of adults (both sexes and males, respectively) treated with agalsidase beta and 24% to 56% of male adults treated with agalsidase alfa developed ADAs,[5,25] with a high incidence of neutralizing antibodies.[26] The presence of neutralizing ADAs has been associated with reduced pharmacodynamic and clinical responses,[25,26] such as severely impaired cardiac structural disease burden and renal outcome, increasing lyso-Gb3 levels, and worse severity score values.[26]

In the present study, three patients (19%) treated with pegunigalsidase alfa developed treatment-induced ADAs, two of which had neutralizing activity. In all cases, the ADA response was transient, with no observed impact on pharmacodynamics, efficacy, or safety. Importantly, no patients treated with the highest dose, 2.0 mg/kg, were ADA-positive at any time.

The pharmacodynamic data demonstrating substantial decrease in Gb3 deposition in kidney peritubular capillaries and a decrease in plasma lyso-Gb3 are predictors of possible clinical benefit of pegunigalsidase alfa in Fabry disease patients.

Progressive deterioration of kidney function is one of the major contributors to morbidity and mortality in classically affected patients with FD.[2,27] Current experience in FD suggests that continuous ERT may slow the progression of chronic kidney disease; however, a subset of treated patients

is characterized by excessive proteinuria (≥1 g/day) or low baseline GFR (<60 mL/min/1.73 m^2) and, as a result, are at high risk for continued loss of renal function.[9,27–29] In the present study with ERT-naïve patients, eGFR was stable during the 1-year treatment period. This observation, combined with the reduction in proteinuria in a small number of patients with baseline UPCR ≥200 mg/g, suggests that pegunigalsidase alfa may stabilize renal function. However, it should be noted that given their normal baseline eGFR and low proteinuria levels, this cohort was already at low risk for loss of eGFR over the study period.

Cardiac manifestations of FD, including left ventricular hypertrophy, myocardial fibrosis, and associated functional deterioration, also contribute to the morbidity and mortality of the disease.[30,31] In this study, a small decrease in LVMi was observed in patients with classic phenotype FD during the 1-year treatment period, although it is important to note that with no fibrosis and normal LVMi prior to treatment in these low-risk patients, no significant improvement would have been expected. Other exploratory clinical outcomes also showed beneficial trends, suggesting the clinical value of pegunigalsidase alfa.

Repeated administration of pegunigalsidase alfa in ERT-naïve male and female patients with FD was well tolerated, and no safety issues unique to this novel ERT for the treatment of FD were observed. Infusion reactions have been reported in ~50% of patients treated with agalsidase beta, necessitating use of antipyretic and antihistaminic premedication.[4] In this study, 24 possible infusion reactions (defined as possibly, probably, or definitely TEAEs occurring during or within 2 hours post-infusion) occurred out of the total of 223 TEAEs. One patient experienced a hypersensitivity reaction (bronchospasm) during the first infusion and was withdrawn from the study per protocol. Premedication was used only in the 2.0-mg/kg group and is being discontinued during the extension study.

6 | STUDY LIMITATIONS

This open-label dose-ranging study was limited by the small number of patients in each dose group, the lack of a control group, and the cohort's relatively mild FD, resulting in limited potential for disease progression.

7 | CONCLUSIONS

The unique pharmacokinetics and the apparent attenuated immune response demonstrated by pegunigalsidase alfa may improve the safety and clinical response to this ERT in patients with FD. This is supported by the favorable AE profile, stability of renal function and cardiac parameters, and improved disease symptoms observed in the study participants during the 12-month treatment period. The clinical efficacy of pegunigalsidase alfa is being further evaluated in three Phase 3 clinical studies.

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CONFLICT OF INTEREST

R.S. reports grant funds, and consultancy and research funds, from Amicus Therapeutics, and consultancy and research funds from Sanofi Genzyme, all outside the submitted work. O.G.-A. reports, along with participation in the current Protalix-sponsored study, grant funds from and use of a product manufactured by Protalix during the conduct of the study; and outside the submitted work, membership on company advisory boards/similar committees for Genzyme, Protalix, and Shire; receiving consulting fees or other remuneration, including speaker fees, from Actelion, Genzyme, Pfizer, and Shire; receiving research support from Alexion, Amicus, Genzyme, Pfizer, Protalix, and Shire; assisting in the design of and/or participating in clinical studies using products manufactured by Genzyme, Protalix, and Shire; and current or recent participation in clinical trials sponsored by Genzyme, Protalix, and Shire. M.H. reports institutional research funding and associated travel reimbursement for scientific meeting presentation from Protalix during the conduct of the study; and outside the submitted work, personal fees and institutional funding for rare disease registry data entry and data presentation from Sanofi-Genzyme. R.B.C. reviewed pathology of trial-coded samples for Protalix during the conduct of the study. K.N. reports nonfinancial support provided by Protalix during the current study; and outside the submitted work, grant funds from Shire Genetic Therapies, and personal fees and nonfinancial support from Shire Genetic Therapies, Genzyme Sanofi, and Amicus Therapeutics. B.R. reports receiving personal fees from Protalix Biotherapeutics during the conduct of the study; and outside the submitted work, personal fees from

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AUTHOR CONTRIBUTIONS

M.H., L.B., A.P., M.S., E.B.-A., R.C., and D.H. conceived and designed this study. R.S., O.G.-A., M.H., P.G., L.B., R.B.C., J.C.J., G.M., S.A.B., D.G., K.N., A.T., M.S., S.A., R.C., and D.H. contributed to the acquisition of data. R.S., M.H., L.B., R.B.C., J.C.J., G.M., S.A.B., M.G.A., B.R., M.R.C., A.P., S.A., E.B.-A., R.C., and D.H. analyzed and interpreted the data. All authors had full access to the data, participated fully in drafting and revising the manuscript, approved the final manuscript, and agreed to submit it to *Journal of Inherited Metabolic Disease.*

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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