23 February 2023
EMA/58745/2023
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Elfabrio

International non-proprietary name: pegunigalsidase alfa

Procedure No. EMEA/H/C/005618/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

ACEi	Angiotensin converting enzyme inhibitors
ADA	Anti-Drug Antibody
ADR	Adverse Drug Reactions
AE	Adverse Event
AET	analytical evaluation threshold
AKI	acute kidney injury
ALT	Alanine aminotransferase
ANCOVA	Analysis of Covariance
ARB	Angiotensin receptor blockers
AST	Aspartate aminotransferase
AUC	Area under the plasma drug concentration-time curve
BCL	Before Cross Link
BDS	bulk drug substance
Bis-NHS-PEG	bifunctional crosslinking reagent N-hydroxysuccinimide-ester activated PEG
BL	Baseline
BLISS	Barisoni Lipid Inclusion Scoring System
BPI	Brief Pain Inventory
BY2	Bright Yellow 2
CHMP	Committee for Evaluation of Human Medicinal Products
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease – Epidemiology Collaboration
C_L	Clearance
C_{max}	maximum observed plasma concentration
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CPP	critical process parameter
CQA	critical quality attributes
CRF	Case Report Form
CSR	Clinical Study Report
DNA	Deoxyribonucleic Acid
E4W	Every 4 Weeks
ECG	Electrocardiogram
ECHA	European Chemical Agency
EF	Ejection Fraction
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-Linked Immunosorbent Assay
Emax	Maximum Effect
EMA	European Medicines Agency
EOW	Every Other Week
EQ-5D- 5L	EuroQol 5 Dimensions 5 Levels Quality-of-life Questionnaire
ERT	Enzyme Replacement Therapy
ESRD	End-stage renal disease
EU	European Union
FCE	Fabry Clinical Events
FD	Fabry Disease
Gb3	glycolipid globotriaosylceramide
GCP	Good Clinical Practices
GD	Gestational day
GLA	gene that encodes the enzyme α-GAL-A
GLP	Good Laboratory Practices
GSA	Gastrointestinal Symptoms Assessment
HCP	Healthcare Professionals
HEENT	head, eyes, ears, nose, throat
HIC	hydrophobic interaction chromatography
IBD	International Birth Date
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G

IP	Investigation Product
IPC	in-process controls
IPTG	Isopropyl β-d-1-thiogalactopyranoside
IRR	Infusion related reactions
ISS	Integrated Summary Safety
ITT	Intent to Treat
IV	intravenous
K_M	Michaelis Menten Constant
LIVCA	limit of in vitro cell age
LL	Lower limit
LOCF	Last Observation Carried Out Forward
LOQ	limit of quantitation
LVM	Left Ventricular Mass
LVMI	Left Ventricular Mass Index
Lyso-Gb3	Globotriaosylsphingosine
M6P	mannose-6-phosphate
MAA	Marketing Authorisation Application
MAR	Missing at random
MCB	master cell bank
MI	Multiple imputation
MMRM	Mixed Model Repeated Measure
MRI	Magnetic resonance imaging
MSSI	Mainz Severity Score Index
n	Number of Patients with Data in the category
N	Number of Patients in the Analysed Population
NA/na	Data not available/Not Applicable
NCA	non-compartmental analysis
NHS	N-hydroxysuccinimide
NI	Non Inferiority
NOR	normal operating range
NYHA	New York Heart Association
PACMP	post approval change management protocol
PAR	proven acceptable range
PBT	Persistent, Bioaccumulative, Toxic
PC	polycarbonate
PCR	polymerase chain reaction
PD	Pharmacodynamics
PDE	permitted daily exposure
PEG	polyethylene glycol
PI	process indicators
PIP	Paediatric Investigation Plan
PK	Pharmacokinetics
pNPG	*para* nitrophenyl-galactoside
PP	Per Protocol
PP	process parameter
PPCO	polypropylene copolymer
PPQ	process performance qualification
prh-alpha-Gal-A	pegylated, recombinant human α-galactosidase-A
PSUR	Periodic Safety Update Report
PT	Preferred Term
PTC	Peri Tubular Capillaries
PVC	polyvinyl chloride
Q2W	Every 2 weeks
Q4W	Every 4 weeks
QoL	Quality of Life
QTPP	quality target product profile
RI	random intercept
RIRS	random intercept random slope
RP-HPLC	reversed-phase high-performance liquid chromatography

RP-HPLC-MS	reverse phase high-performance liquid chromatography-mass spectrometry
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDS-PAGE	sodium dodecyl-sulfate polyacrylamide gel electrophoresis
SE	Standard Error
SEC	size exclusion chromatography
SEC-MALS	multi-angle light scattering coupled with size exclusion chromatography
SE-HPLC	size exclusion-high-performance liquid chromatography
SmPC	Summary of Product Characteristics
SOC	System Organ Class
$T_{1/2}$	Half-life
TEAE	Treatment emergent adverse event
TMV	Tobacco Mosaic Virus
TTC	Threshold of Toxicological Concern
UL	Upper Limit
ULN	Upper Limit of Normal
UPCR	Urine protein to creatinine ratio
V_{ss}	Steady state volume
Vz	Terminal phase volume
WCB	working cell bank
WFI	water for injections
α-Gal-A	α-galactosidase-A

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Chiesi Farmaceutici S.p.A. submitted on 25 January 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Elfabrio, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 March 2020.

Elfabrio, was designated as an orphan medicinal product EU/3/17/1953 on 12 December 2017 in the following condition: Treatment of Fabry disease.

Following the CHMP positive opinion on this marketing authorisation and at the time of the review of the orphan designation by the Committee for Orphan Medicinal Products (COMP), this product was withdrawn from the Community Register of designated orphan medicinal products on 24 March 2023 on request of the sponsor. The relevant orphan designation withdrawal assessment report can be found under the 'Assessment history' tab on the Agency's website https://www.ema.europa.eu/en/medicines/human/EPAR/Elfabrio.

The applicant applied for the following indication:

Elfabrio is indicated for long-term enzyme replacement therapy in adult patients with a confirmed diagnosis of Fabry disease (deficiency of alpha-galactosidase).

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0109/2016 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0109/2016 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. New active Substance status

At initial submission, the applicant requested the active substance pegunigalsidase alfa contained in the above medicinal product to be considered as a new active substance.

On 18 January 2023, the applicant withdrew their claim for new active substance.

1.6. Protocol assistance

The applicant received the following Scientific Advice on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
17 December 2015	EMEA/H/SA/3189/1/2015/SME/III	*Dr Amany N. El-Gazayerly and Dr Karl-Heinz Huemer*

The Scientific Advice pertained to the following quality, non-clinical, and clinical aspects:

Quality:

- Product characterisation, control of impurities, and in-process controls for the active substance manufacture.
- Release and stability testing of the active substance and finished product.
- Omission of virus and mycoplasma testing in view of the use of a plant manufacturing system.
- MCB and WCB characterisation.

Non-Clinical:

- Adequacy of the non-clinical studies to support a MAA.

Clinical:

- Agreement that the Fabry disease adult population naïve to ERT are not assessed in Phase 3 clinical studies, but only within the Phase 1/2 clinical trial for a total treatment duration of 3 years.
- Agreement that the Phase 1/2 clinical study will provide sufficient pharmacokinetic data for a MAA.
- Acceptability of the first of two proposed switch-over Phase 3 studies to support a MAA, in particular with regards to the overall study duration including interim report, primary and secondary endpoint and sample size.
- Statistical basis of the head-to-head study, Elfabrio versus Fabrazyme.
- Acceptability of the second of two proposed switch-over Phase 3 studies to support a MAA, in particular with regards to the overall study duration, study design, endpoints and sample size.
- Agreement that the two switch-over Phase 3 clinical studies, together with the Phase 1/2 studies in the ERT treatment-naïve adult population, would suffice for assessment of safety and efficacy, for a MAA.
- Clinical immunogenicity testing approach.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Alexandre Moreau Co-Rapporteur: Robert Porszasz

The Rapporteur appointed by the PRAC was:

PRAC Rapporteur: Liana Gross-Martirosyan

The application was received by the EMA on	25 January 2022
The procedure started on	24 February 2022
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 May 2022
The CHMP Co-Rapporteur's critique was circulated to all CHMP and PRAC members on	30 May 2022
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	31 May 2022
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	23 June 2022
The applicant submitted the responses to the CHMP consolidated List of Questions on	09 September 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	21 October 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	27 October 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	3 November 2022
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	10 November 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	16 December 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	20 January 2023
The CHMP agreed on a list of outstanding issues in writing to be sent to the applicant on	26 January 2023

The applicant submitted the responses to the CHMP Second List of Outstanding Issues on	31 January 2023
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	17 February 2023
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Elfabrio on	23 February 2023
The CHMP adopted a report on similarity of Elfabrio with Galafold on (see Appendix on similarity)	26 January 2023

2. Scientific discussion

2.1. Problem statement

Pegunigalsidase alfa (also referred to as PRX-102) is a form of human α-galactosidase-A (α-Gal-A), produced in tobacco cells (Nicotiana tabacum BY2 cells) using recombinant DNA technology. which has been chemically modified by short (2.3 KDa) bis-NHS-PEG reagents to provide PEGylated monomers, the majority of which are cross-linked to give covalently bound homodimer molecules through PEG chains. It is a product intended for the treatment of adult patients with Fabry disease.

2.1.1. Disease or condition

Fabry disease is a debilitating progressive lysosomal storage disorder characterized by subnormal or absent activity of alpha-galactosidase A, a lysosomal enzyme that primarily catalyses the hydrolysis of the glycolipid globotriaosylceramide, Gb3, to galactose and lactosylceramide.

2.1.2. Epidemiology

Fabry disease is an X-linked disorder caused by alterations in the gene that encodes the enzyme α-GAL-A (GLA) leading to deficient/absent enzymatic activity and resulting in glycosphingolipid accumulation with life-threatening complications [Ortiz 2018]. It is regarded as a rare disease and it was originally estimated that 1 in 40,000 males has the disease, whereas the estimated prevalence in the general population was 1 in 117,000 [Meikle 1999]. However, newer studies suggest that Fabry disease has a much higher prevalence following the development of better diagnostic tools and increasing awareness [Hoffmann 2009]. Due to the nonspecific nature of the clinical manifestations of Fabry disease and the common occurrence of a single complication, it is likely that many undiagnosed patients exist, which explains the discrepancy in frequency.

Furthermore, the presence of equal numbers of females and males in large populations studied suggests that up to 50% of the females with Fabry disease may not have been identified [Eng 2007; Mehta 2004]. An α-GAL-A enzyme activity screening of 37,104 consecutive newborn males confirmed by sequencing of genomic DNA indicated the prevalence for Fabry Disease in Italy to be 1 in 3,100 males [Spada 2006]. This study identified and included often undiagnosed patients with the non-classic form of Fabry disease; the ratio of non-classic to classic was determined to be 11 to 1 [Spada 2006]. A random screening of 110,027 newborns in Taiwan discovered the incidence in males to be about 1 in 1,500 [Lin 2009]. This study also determined there

were still significantly fewer females affected than males, with approximately 1 in 17,000 affected; however, this prevalence is still much higher than was previously thought for males [Lin 2009]. Screening for Fabry disease reveals a high prevalence of individuals with GLA genetic variants of unknown significance. The prevalence of GLA variants in newborns was found to be 0.04%, while in high-risk populations, the overall prevalence of individuals with GLA variants was 0.62%, with the prevalence of a definite diagnosis of Fabry disease as 0.12% [van der Tol 2016].

2.1.3. Biologic features, Aetiology and pathogenesis

Fabry disease is characterised by mutations in the GLA gene, resulting in decreased/undetectable levels of α-Gal-A activity in plasma or leukocytes, typically observed together, with high concentrations of the substrate Gb3 and its degradation product, Lyso-Gb3 in tissues and plasma, which both correlate with organ damage [Aerts 2008; Boutin 2014; Ouyang 2017; Kramer 2018].

Progressive accumulation of Gb3, Lyso-Gb3, and related lipids, leads to impaired tissue and organ function, particularly in the kidney, heart, and cerebrovascular system [Aerts 2008; Schiffmann 2009]. In addition, involvement of the central, peripheral, and autonomic nervous systems result in episodes of pain and impaired peripheral sensation.

A recent review by Kramer and Weidmann surveyed 70 peer-reviewed publications regarding Fabry specific biomarkers and identified Lyso-Gb3 as a biomarker with clinical applicability. Reductions of plasma Lyso-Gb3 were found to be indicative of treatment response, i.e., of ERT performance [Kramer 2018]. Furthermore, when ADAs to ERT form, they decrease the ability of ERT to reduce plasma Lyso-Gb3 concentration [Sakuraba 2018; Mauhin 2018; Rombach 2012].

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Fabry disease is typically categorised using two subtypes, classic and non-classic, based on age of symptom onset and extent of organ involvement. Clinical onset of the classic form of disease typically occurs during childhood or adolescence [Schaefer 2009] and progresses to end-stage renal disease (ESRD), cardiac complications, and/or cerebrovascular disease in the fourth or fifth decade of life [Branton 2002]. Non-classical Fabry disease, also referred to as late-onset or atypical disease, is characterised by a more variable disease course, in which patients are generally less severely affected and disease manifestations may be limited to a single organ. Males with nonclassical disease typically have >5% residual enzyme activity and lower levels of the deacetylated substrate [Lyso-Gb3; Arends 2017].

Classic Fabry Disease

The discussion and parameters regarding the definition of a classic Fabry disease patient is evolving and, in some cases, challenging. Currently, there is no consensus in the published literature regarding the definition of classic Fabry disease in either males or females [Arends 2017; van der Tol 2014; Mehta 2002; Smid 2013; Chien 2012; Nakao 2003; Salviati 2010]. Patients were considered to be classic when the following criteria were met: (1) a GLA disease-causing mutation, (2) enzyme activity ≤5% of the mean reference range, and (3) one or more characteristic Fabry disease symptoms (i.e., Fabry neuropathic pain, angiokeratoma, and/or cornea verticillata).

Disease Manifestation in Males

Primary clinical manifestations of Fabry Disease in males are neuropathic pain and acroparaesthesia; angiokeratomas; hypohidrosis; cornea verticillata; hearing loss; and gastrointestinal symptoms. Renal

disease is associated with progressive proteinuria accompanied by a decline in eGFR, leading over a number of years to ESRD requiring dialysis and/or kidney transplantation [Branton 2002; Ortiz 2008]. There is evidence that hyperfiltration often precedes the decline in renal function, particularly in childhood; hyperfiltration will present as greater than normal estimated glomerular filtration rate (eGFR) but represents evolving glomerulopathy [Ries 2005; Vedder 2007].

Cardiac disease is associated with progressive hypertrophic cardiomyopathy with diastolic dysfunction, a variety of conduction defects and arrhythmias such as short P–R interval, and supraventricular and ventricular tachycardia. Other complications are valvular disease (insufficiency or stenosis) and coronary artery stenosis of large or, more commonly, of small vessels [Kampmann 2008; Weidemann 2005]. Progressive bradycardia and decreased exercise capacity are also very common [Lobo 2008].

It has been estimated that patients with Fabry disease have a 20-fold increased risk of ischemic stroke and transient ischemic attacks compared to the general population. Both small and large vessel strokes occur, with brain regions perfused by the posterior circulation being affected more commonly than anterior circulation [Moore 2001; Moore 2003].

Manifestation in Females

Due to the X-linked nature of the disease, males are hemizygotes and females are heterozygotes and affected by the mosaicism effect [Hughes 2011]. It is now widely accepted that females may express a range of clinical features including life-threatening manifestations such as cardiomyopathy, renal disease and stroke [MacDermot 2001; Whybra 2009; Sunder-Plassmann 2006]. There is considerable variation in the phenotype in heterozygous females. Age has the strongest correlation with the presence of symptoms, with the proportion of women suffering from a particular symptom manifesting approximately a decade after that symptom is found in males. The median age of onset of symptoms is 13 years in female as compared to 9 years in males [Hughes 2011]. Only 7.6% of females were diagnosed prior to symptom onset, largely due to screening of families. Forty-three percent (43%) of diagnosed females are receiving ERT with median age of initiation of 44.8 years.

2.1.5. Management

There are currently two approved classes of therapies available for patients with Fabry disease, ERT (Fabrazyme and Replagal) and pharmacological chaperone (Galafold).

Fabrazyme (agalsidase beta 1.0 mg/kg EOW) and Replagal (agalsidase alfa 0.2 mg/kg EOW) are ERTs authorised in the EU and administered by IV infusion. ERT is the longest and most successfully employed drug treatment for lysosomal storage disorders indicated across the entire spectrum of disease-causing mutations.

Both enzymes have shown effects in clinical studies with regard to the preservation of renal function [Hughes 2017; Vedder 2007; Banikazemi 2007; Eng 2001; Ortiz 2021; Wanner 2020; Germain 2007; Germain 2010; Germain 2016; Mehta 2010; Wilcox 2004; Schiffmann 2006; Schiffmann 2009]. In addition, some improvement in clearance of Gb3 from kidney cells (such as capillary endothelial cells, glomerular endothelial cells, noncapillary endothelial cells and noncapillary smooth muscle cells), and capillary endothelial cells of the myocardium and skin plasma [Eng 2001; Germain 2007; Schaefer 2009] as well as quality of life (QoL), reduction or stabilisation of cardiac mass can also be observed.

However, despite the beneficial effects described above, Fabry disease patients generally show only limited clinical improvement on the current ERTs.

Galafold (migalastat) is the only approved molecular chaperone and is only effective for a subgroup of ~30% of Fabry disease patients with amenable mutations. Galafold is approved in the United States, European Union and other countries based on reduction of renal Gb3 inclusions.

2.2. About the product

Pegunigalsidase alfa is a recombinant human α-Gal-A produced in cultured tobacco (BY2) cells and PEGylated to become a covalently linked homodimer. The production in plant cells results in mannose terminated glycosylation [Kizhner 2015] that targets pegunigalsidase alfa to disease-relevant tissues, and the PEGylation is claimed to decrease the immunogenicity and further enhance enzyme exposure to target organs, thus improving PK properties.

Pegunigalsidase alfa is categorised as an ERT to supplement biological active α-GAL-A enzyme to Fabry disease patients characterised by deficient/absent enzyme activity. This deficiency/absence results in massive storage of Gb3 and related glycolipids (e.g., Lyso-Gb3) in cells of the vascular system, cardiomyocytes, neuronal cells and kidney cells as well as elevated levels in the circulation. The ultimate consequence of glycosphingolipid deposition in the vasculature and other tissues is end-organ failure, particularly of the kidney, but also of the cardiac and cerebrovascular system [Schiffmann 2009].

2.3. Type of Application and aspects on development

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as concentrate for solution for infusion containing 2 mg/mL pegunigalsidase alfa as active substance. Other ingredients are: sodium citrate tribasic dihydrate, citric acid, sodium chloride and water for injections (WFI). The product is available in a 10 ml vial (15R clear glass) closed with coated rubber stopper and sealed with aluminium flip off cap. Pack sizes are 1, 5 or 10 vials.

Prior to administration, the required amount of pegunigalsidase alfa finished product is added to the infusion bag containing sterile 0.9% sodium chloride solution and administered via intravenous (IV) infusion. No diluent is accompanying the finished product.

2.4.2. Active Substance

2.4.2.1. General Information

Pegunigalsidase alfa (INN), the active substance contained in Elfabrio is a recombinant, pegylated form of human α-galactosidase-A (prh-alpha-Gal-A). It is produced in tobacco cells (*Nicotiana tabacum* BY2 cells) using recombinant DNA technology.

The amino acid sequence of prh-alpha-GAL-A is pictured as follows (

1	GLDNGLARTP	TMGWLHWERF	MCNLDCQEEP	DSCISEKLFM	EMAELMVSEG
51	WKDAGYEYLC	IDDCWMAPQR	DSEGRLQADP	QRFPHGIRQL	ANYVHSKGLK
101	LGIYADVGNK	TCAGFPGSFG	YYDIDAQTFA	DWGVDLLKFD	GCYCDSLENL
151	ADGYKHMSLA	LNRTGRSIVY	SCEWPLYMWP	FQKPNYTEIR	QYCNHWRNFA
201	DIDDSWKSIK	SILDWTSFNQ	ERIVDVAGPG	GWNDPDMLVI	GNFGLSWNQQ
251	VTQMALWAIM	AAPLFMSNDL	RHISPQAKAL	LQDKDVIAIN	QDPLGKQGYQ

301	LRQGDNFEVW	ERPLSGLAWA	VAMINRQEIG	GPRSYTIAVA	SLGKGVACNP
351	ACFITQLLPV	KRKLGFYEWT	SRLRSHINPT	GTVLLQLENT	MQMSLKDLLS
401	EKDEL				

Figure 1):

1	GLDNGLARTP	TMGWLHWERF	MCNLDCQEEP	DSCISEKLFM	EMAELMVSEG
51	WKDAGYEYLC	IDDCWMAPQR	DSEGRLQADP	QRFPHGIRQL	ANYVHSKGLK
101	LGIYADVGNK	TCAGFPGSFG	YYDIDAQTFA	DWGVDLLKFD	GCYCDSLENL
151	ADGYKHMSLA	LNRTGRSIVY	SCEWPLYMWP	FQKPNYTEIR	QYCNHWRNFA
201	DIDDSWKSIK	SILDWTSFNQ	ERIVDVAGPG	GWNDPDMLVI	GNFGLSWNQQ
251	VTQMALWAIM	AAPLFMSNDL	RHISPQAKAL	LQDKDVIAIN	QDPLGKQGYQ
301	LRQGDNFEVW	ERPLSGLAWA	VAMINRQEIG	GPRSYTIAVA	SLGKGVACNP
351	ACFITQLLPV	KRKLGFYEWT	SRLRSHINPT	GTVLLQLENT	MQMSLKDLLS
401	EKDEL				

Figure 1 Pegunigalsidase alfa amino acid sequence

The majority of pegylated prh-alpha-GAL-A monomers are cross-linked to a covalently bound homodimer, some of the polyethylene glycol (PEG) moieties are bound to one monomer only. Pegunigalsidase alfa contains also noncovalently bound homodimers when no crosslinking occurs. All covalently and non-covalently bound pegylated homodimers are biologically active. In addition, biologically active tetramers, i.e., two homodimers are also found.

The theoretical molecular weight is 46,110 Da. The molecular weight of pegunigalsidase homodimer comprising two pegylated alpha-GAL-A subunits is approximately 116 kDa.

The pegylation is achieved with a bis-NHS-PEG reagent (NHS represents N-hydroxysuccinimide) containing amino-groups substituted with succinic acid, yielding a crosslinker with an average total mass of 2300 Da. An average of 8-9 PEG moieties are reported per monomer.

To be biologically active, pegunigalsidase is internalized to lysosomes where it hydrolyses terminal alpha-galactosyl moieties from the glycolipid globotriaosylceramide (Gb3) to yield galactose and lactosylceramide.

2.4.2.2. Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The active substance is manufactured at Protalix Ltd, Israel. A valid GMP certificate is available.

Recombinant pegunigalsidase alfa active substance is manufactured using a genetically modified Tobacco BY-2 plant cell line. The manufacturing process includes an upstream (“production train”) and downstream purification processing stepsError! Reference source not found.

Upstream processing starts with the thaw of working cell bank (WCB) and cell expansion through petri dishes, shake flasks and bioreactors of increasing size. The cell content of “inoculum” bioreactors, are used for biomass production for inoculation of the “induction” bioreactors.

The cells in the “induction” bioreactors express the prh alpha-GAL-A protein. The pooled harvests of bioreactors are separated and the soluble prh alpha-GAL-A protein is extracted and clarified by filtration and purification using chromatographic columns to produce the intermediate, non-pegylated product designated as BCL (Before Cross Link). The BCL intermediate may be stored at adequate conditions.

Downstream process continues with thawing BCL intermediate batches to conduct the chemical cross-link reaction. The bifunctional crosslinking reagent N-hydroxysuccinimide-ester activated PEG (Bis-NHS-PEG) is used to generate pegylated prh alpha-GAL-A protein. To purify the cross-linked prh alpha-GAL-A protein, a final chromatographic column is used, which is followed by compounding to a final concentration of 2 mg/mL

pegunigalsidase. The solution is filtered into polycarbonate (PC) bottles with PP screw caps for storage at 2-8°C for not more than 5 days before freezing.

Pegunigalsidase is stored at -70 ±15°C.

2.

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Pegunigalsidase is stored at -70 ±15°C.

2*Control of materials*

Information on grade and quality of the raw materials used during upstream/downstream processing is acceptable. The composition of culture media is described. Filter and resin types as well as buffer compositions are listed in the dossier. No raw materials of direct animal or human origin are used during the manufacturing process.

The generation of the cell substrate is described in detail. A plant cell line, *Nicotiana tabacum cv. Bright Yellow 2* (BY2) was chosen for transformation with the human alpha-GAL-A transgene. The generation of the cell banks is described. The master cell bank (MCB) and WCB were characterised by testing for sterility, cell viability, cell growth at harvest, alpha GAL-A protein expression. In general characterisation for MCB/WCB is considered acceptable. Additional information on identity testing as well as genetic stability testing to confirm insert coding sequence in the WCB and/or the limit of in vitro cell age (LIVCA) cells is provided. The procedures for preparing a new WCB as well as the requirements for its characterisation are described and considered acceptable.

Nucleic acid sequencing of the alpha-Gal-A insert in cells cultured up to the LIVCA under conditions representative for the manufacturing scale are provided to demonstrate stability up to the maximum harvest culture time. Calculation of the maximum culture duration claimed for the LIVCA is described and is considered adequate.

Control of critical steps and intermediates

Acceptable process parameter and controls including process parameter (PP) and critical process parameter (CPP) as well as process indicators (PI) and in-process controls (IPC) are implemented together with their acceptance criteria (Normal Operating Range, NOR). Proven acceptable ranges (PAR) are provided as well. Acceptable bioburden and endotoxin control is integrated in both upstream and downstream procedures. Both process-related and product-related in-process controls have been established for the downstream procedure. The critical steps as well as the control parameter for pegunigalsidase alfa active substance manufacturing process have been defined using a risk mitigation approach. This is considered acceptable.

Overall, well-structured information has been provided for the active substance manufacturing process and its control. The provided batch definition is considered reasonable.

BCL (before cross linking) intermediate

An acceptable specification has been provided for BCL intermediateError! Reference source not found.. Analytical procedures included in the BCL intermediate specification are adequately described and respective method validation data are considered appropriate.

Activated PEG intermediate

In the initial submission activated PEG intermediate was classified as starting material. However, PEG should be classified as a starting material and the activated PEG moiety should be defined as intermediate, as the activation of PEG is considered part of the manufacturing process. A major objection was raised on this point. As requested, activated PEG has subsequently been re-classified as an intermediate, while the PEG used to produce the activated PEG has been classified as a starting material. Appropriate and sufficient information is provided with regard to the PEG starting material, the manufacturing process and its control as well as process-related impurities. Necessary controls have been implemented into the activated PEG specification. Acceptable description and qualification of analytical test methods used for control of activated PEG intermediate is provided as well as stability data supporting the assigned shelf life. Moreover, it is confirmed that the PEG is processed to activated PEG under GMP conditions. Since the PEG intermediate is a chemically defined material, this is considered appropriate.

1*Process validation*

Process verification has been performed by manufacture of batches of BCL intermediate originating from different “production trains” with active substance batches. All were manufactured with the commercial scale and process.

Process validation/process performance qualification (PPQ) studies have been performed. The choice of batches and overall setting for process verification seems reasonable.

Generally, all input and output parameters including PPs, CPPs, PI and IPCs met the pre-defined acceptance criteria and based on that, in general, the active substance manufacturing process can be considered in a validated state. In few cases, input and output parameter were found deviating from pre-defined acceptance criteria. After root cause analysis, acceptance criteria have been updated which is covered by GMP.

Resin lifetime of the chromatography columns used during the active substance manufacturing process has been defined. For resin lifetime evaluation at commercial scale, acceptable protocols are provided.

During active substance manufacture, a number of disposable bags and bioreactors are used. Risk assessment is performed to investigate potential extractables and leachables in the active substance. However, the analytical investigation on leachables was conducted at the finished product level.

Manufacturing process development

Development of pegunigalsidase alfa manufacturing process was performed at Protalix Ltd., Israel using a proprietary bioreactor system and plant cell technology and taking into consideration prior knowledge gained for Elelyso (taliglucerase alfa).

Different manufacturing processes phases have been described. In general, the differences between the process phases have been described with sufficient detail. Comparability between the different process materials has been adequately addressed with reference to release and stability data available for BCL intermediate and active substance. Overall, comparable data are available for the quality attributes of the pre-change and post-change materials including the commercial process material demonstrating representativeness of the clinical materials for the commercial scale materials.

To manufacture pegunigalsidase alfa active substance, critical quality attributes were identified and a process control strategy was developed. Implementation of the control strategy seems reasonable and is considered acceptable. Classification of process input and process output parameter into critical process parameter (CPP)/ IPCs and other process parameter (PP)/ process indicators (PI) is considered adequate. Proven acceptable ranges for process parameter have been confirmed using lab-scale, pilot-scale and commercial scale studies challenging the edges of PAR(s). The applied small-scale models seem representative for the commercial scale since operating parameter were kept the same. Parameters that were found to critically affect either process performance or product quality were defined as CPP. This is considered acceptable.

The process step of PEG cross linking and its development/optimisation is specifically addressed.

Crosslinking using a bis-pegylating reagent aims to increase active substance stability and thus to retain enzymatic activity. In the course of development, different pegylation reagents with different qualities have been used to produce pre-clinical, clinical and commercial batches. The impact of these quality differences on the resulting active substance batches used during pre-clinical studies, clinical studies and for commercial manufacture has been evaluated. Effectivity of the PEG crosslinking is adequately monitored by appropriate techniques.

The presented data support comparability of the active substance materials manufactured with different pegylation reagents of different qualities. Likewise, the presented data support the Applicant's conclusion that the impact of these quality differences on the resulting active substance batches used during pre-clinical studies, clinical studies and for commercial manufacture can be neglected.

Characterisation

Pegunigasidase alfa is a complex molecule, consisting of the homodimeric protein part of alpha galactosidase. The structure was adequately characterised and confirmed and the biological activity adequately studied and demonstrated as well.

In conclusion, the active substance has been sufficiently characterised.

Impurities

The Applicant provided a very comprehensive evaluation of impurities (potentially) present in pegunigalsidase active substance. Evaluation included an assessment where impurities originate from, capability of analytical methods to detect such impurities, and clearance studies for the process-related impurities at different scales. Forced degradation studies were performed to identify degradation products and elucidate degradation pathways. Further, these studies supported the stability indicating potential of the analytical methods used.

Impurity levels have been qualified by nonclinical and clinical studies as relevant.

Product-related impurities were already extensively discussed in the characterisation section. They are addressed by suitable methods; for some of them specifications are established in the active substance specification.

For evaluation of process-related impurities, sufficient clearance to acceptable levels could be demonstrated.

The clearance capability of the manufacturing process was repeated at production scale (where no spiking is intended) to support robustness of the process. Worst case conditions were chosen where deemed valuable. Similarly, it could be demonstrated that the process is capable to reduce impurities to either insignificant levels or to levels well within the proposed limit specification at release of intermediate/active substance.

Forced degradation studies were conducted to analyse the effects of stress on product degradation and to identify and evaluate degradation kinetics and pathways. The study design comprised several levels where batches, stress conditions, and methods to analyse degradation were varied.

Overall, valuable information on degradation, degradation pathways and reaction kinetics was obtained by the various forced degradation studies performed. Thus, the forced degradation studies further enlarged understanding of the quality profile of the active substance.

2.4.2.3. Specification

Active substance

The release and shelf-life specifications for pegunigalsidase alfa active substance 2cover tests for appearance, content and potency, identity, purity and impurities, and general tests.

2In general, an acceptable specification has been provided for the pegunigalsidase active substance. It comprises acceptance criteria for appearance, protein content by UV280 absorbance and determination of enzyme activity using enzyme-substrate *in vitro* test as well as potency testing using cellular uptake assay. Identity is determined using peptide map, SDS-PAGE/ Western Blot and molecular weight analysis. Purity is controlled by SE-HPLC (denaturing and non-denaturing), peptide map (RP-HPLC), free sulfhydryl content, amine site occupancy and total PEG content. pH, total aerobic count and bacterial endotoxins are controlled as general tests.

The proposed specifications are considered acceptable.

Analytical methods

Detailed descriptions of analytical methods have been provided for the release methods as well as the respective method codes. Analytical method validation summaries provided are found acceptable.

Reference materials

Reference standards have been established for control of BCL intermediate and for control of active substance/finished product. Qualification of reference standards used in the past is described in sufficient detail. For the commercial process, a primary reference standard as well as working reference standard have been established for the BCL intermediate and for the active substance.

Qualification of reference standards has been performed based on release testing and additional characterisation. This is considered acceptable.

For establishment and qualification of future working standards, acceptable protocols have been provided. Stability of reference standard materials is also appropriately addressed.

Batch analysis

An extensive release data base is available, including batch information for all active substance batches manufactured so far. Generally, batch data are found complying with the specification being in place at the time of analysis.

Container closure

Sufficient information has been provided on the container closure system used for active substance storage.

2.4.2.4. Stability

A comprehensive data package is provided for stability. Primary stability studies cover commercial active substance batches and supportive stability studies cover active substance batches manufactured with previous process versions.

The primary stability studies were performed in line with ICH guidelines and covered long-term conditions and accelerated conditions. The supportive stability studies also include storage under stress conditions. Forced-degradation studies are described in the characterisation section.

The only differences in the stability specification compared to the release specification are skipped parameters and slightly extended limits for one impurity parameter. The analytical procedures applied to testing of the stability samples are identical to those used for release.

Overall, the stability protocol is acceptable and the chosen analytical procedures are considered stability-indicating. The container closure system used for the stability studies is a smaller size but intended to mimic the commercial container closure system.

The stability data demonstrate the active substance being stable under the proposed storage conditions. All parameters remain within the pre-defined specification limits.

Accelerated stability data is available for all primary stability batches. All parameters remain well within the specifications throughout the duration of the stability studies.

Based on the provided data the proposed shelf of 60 months at -70 °C is considered acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and Pharmaceutical Development

The finished product is presented in 10 mL vial (15R clear glass) vials as concentrate for solution for infusion. The vials are closed with coated rubber stoppers and secured with aluminium flip off caps.

Vials are filled with a sterile citrate buffered solution (pH 5.9-6.4) of 2 mg/mL pegunigalsidase alfa containing 0.7% sodium chloride. The nominal quantity is 20 mg of pegunigalsidase alfa based on an extractable volume of 10 mL per vial. There is an overfill to facilitate extraction of the declared volume.

Prior to administration, the required amount of pegunigalsidase alfa finished product is added to the infusion bag containing sterile 0.9% sodium chloride solution and administered via IV infusion. The diluent is not accompanying the finished product.

The components of the finished product are Pegunigalsidase alfa, Sodium citrate tribasic dihydrate, Citric acid, Sodium chloride. Error! Reference source not found.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation.

The pharmaceutical development of the finished product was based on a quality target product profile (QTPP). Formulation and manufacturing process development took into account the applicable critical quality attributes (CQAs) for a sterile solution in vials.

The pegunigalsidase alfa active substance is an aqueous solution containing 2 mg/mL of pegunigalsidase alfa in sodium citrate buffer with sodium chloride. The finished product is a concentrate for solution for infusion with a physiologically compatible pH (5.9 – 6.4) and osmolality.

Formulation development started based on existing formulations for commercial enzyme replacement products. Lyophilization and liquid formulations were evaluated. The lyophilized formulation was significantly less stable than liquid formulations and was not pursued further. Liquid formulations with different excipients were evaluated resulting in a formulation used for non-clinical trials.

Changes brought throughout the finished product development were adequately described by the Applicant. Subsequent development led to the final clinical formulation that is stored at 2 °C to 8 °C.

Finished product with active substance manufactured from different processes was used in clinical trials phase 1/2 and 3.

Comparability is discussed in the active substance section.

The finished product manufacturing process comprises pooling of active substance, pre-filtration, sterile filtration, aseptic filling, stoppering and crimping of the vials. The Applicant considers the manufacturing process a straightforward process with no need to perform process development, which is accepted. A summary of the risk assessment for defining non-CPPs, CPPs and IPCs of the finished product manufacturing process is provided including justification for all acceptance criteria and PARs.

Container closure

The container closure system consists of clear 15R glass vials closed with rubber stoppers, which are capped with aluminium crimp seals. The specifications for vial and stopper as well as technical drawings and compliance certificates are acceptable. The suitability of the container closure system with respect to chemical compatibility and container closure integrity throughout shelf life is demonstrated by stability data.

The finished product is diluted with 0.9% sterile sodium chloride solution before administration. In-use stability and compatibility with IV bags and infusion sets are discussed in the stability section.

2.4.3.2. Manufacture of the product and process controls

Chiesi Farmaceutici SpA, 96 via S. Leonardo, 43122 Parma, Italy is responsible for secondary packaging and batch release. Proof of GMP compliance for all manufacturing and testing sites is available.

The batch size has been adequately presented.

A flow diagram for the manufacturing process has been presented.

Process parameters, critical process parameters and IPCs as well as process and hold times with acceptance criteria and PARs have been presented for all manufacturing steps. Sterilisation and depyrogenation of the primary packaging components is also described

Process validation / verification

No deviations occurred during the three PPQ runs that impacted CPPs, IPCs and the release testing results. All acceptance criteria for the three PPQ batches were met. Process qualification is considered sufficiently demonstrated. The PPQ runs covered minimum and maximum commercial batch sizes. Two additional batches were utilised for process and hold time validation supporting the defined process and hold times.

Maximum filling time, filter use time, sterilisation and depyrogenation conditions have all been adequately validated.

Shipment of the vials was validated with shipments to the testing site Protalix (Israel). Analytical data has been provided demonstrating that shipment conditions have no detrimental impact on finished product quality.

2.4.3.1. Product specification

The release and shelf-life specifications for the finished product Error! Reference source not found.cover tests for appearance, content and potency, identity, purity and impurities, and general tests.

The finished product release and shelf-life specifications include all required tests, including procedure codes for non-compendial test procedures, for a sterile aqueous finished product containing a protein supplied in a vial.

The batch results indicate that no degradation occurs due to the manufacturing process of the finished product, which does not use steps or conditions considered to have a strong influence on the impurity profile of the finished product.

A risk assessment and corresponding studies on potential leachables and elemental impurities from the manufacturing process equipment and container closure system in the finished product are presented. One batch manufactured at worst case conditions was tested. All leachables and elemental impurities above the analytical evaluation threshold (AET) were evaluated. This approach is considered acceptable.

The applicant provided a toxicological evaluation of the results of potential leachables identified above the AET and concluded that no potential harm is expected from the identified compounds at the levels detected. The evaluation is accepted.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D (R1). Based on the risk assessment and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the “Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products” (EMA/409815/2020) and the “Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products” (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Analytical methods

Finished product specific test procedures are sufficiently described and validated (in-house procedures) or verified (compendial procedures).

Reference materials

The same reference standards are used for both active substance and finished product. Reference is therefore made to the active substance section.

Batch analysis

Release results for batches of finished product have been provided covering clinical and PPQ lots manufactured with the commercial process. Based on the batch results it can be concluded that the manufacturing process is under control and results in consistent and uniform finished product.

2.4.3.2. Stability of the product

Primary stability batches of finished product have been included in the current stability study. The stability studies are carried out in accordance with ICH Q1A (R2).

The shelf-life specification evolved over time, the rationale for the changes is given. The test methods used are the same as those used for release of finished product.

The primary stability batches are stable under long-term conditions for 48 months with all results within the specification limits and no significant trend.

Stability data of the primary stability batches at accelerated storage conditions are within specifications over the studied time period showing only a trend towards a slight decrease in homodimer content.

Data from supportive stability batches are in accordance with the stability data of the primary stability batches.

A photostability study in accordance with ICH Q1B demonstrated that the finished product is not susceptible to light. Furthermore, the applicant demonstrated that short-term thermal excursions (up to 25 °C or down to -20 °C) from the recommended storage conditions (2-8°C) have no negative impact on finished product quality.

The applicant also performed a mechanical stress study to evaluate the impact of agitation of the finished product. Samples were subjected to vertical and horizontal agitation over 7 days. The study showed that mechanical stress leads to large protein aggregates which can be observed visually. Furthermore, the number of subvisible particles increases. These findings are appropriately reflected in the SmPC: “Visually inspect the vials. […] Do not use if there is particulate matter or if it is discoloured. Avoid shaking or agitating the vials.” Furthermore, the diluted finished product should be administered via IV infusion filtered through an in-line low protein-binding 0.2 µm filter.

As part of an in-use study after dilution of the finished product with 0.9% NaCl solution the applicant demonstrated that the application of an in-line low protein binding 0.2 µm filter does not significantly impact appearance, specific activity and protein content of the diluted finished product solution. The in-use study also demonstrated that the diluted finished product solution in polyvinyl chloride (PVC) and non-PVC infusion bags is stable for up to 72 hours at 5±3 °C and at 25±2 °C. For microbiological reasons the in-use stability is reduced in the SmPC:

“From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours in the refrigerator (2 °C-8 °C) or 8 hours if stored below 25 °C, unless dilution has taken place in controlled and validated aseptic conditions.”

Altogether, the proposed shelf-life of the finished product of 4 years at 2-8°C is acceptable.

2.4.3.3. Post approval change management protocol(s)

A post approval change management protocol (PACMP) has been provided. Overall, the PACMP is sufficiently detailed and is therefore considered acceptable.

2.4.3.4. Adventitious agents

No animal-derived materials are used during manufacturing. The production cell line is of plant origin (*Nicotiana tabacum).* Extensive testing by PCR for viruses with the potential to infect tobacco plants has been performed on MCB/WCB, demonstrating that the cell substrate is free from the tested viruses. The information provided is considered sufficient to demonstrate viral safety of the cell substrate. As requested the applicant has clarified that there is no risk of accidental introduction of plant viruses during manufacturing, as plant cell infection relies on transmission by a (insect) vector. Furthermore spreading of plant viruses is hampered due to the disruption of plasmodemata (naturally connecting cytoplasma of adjacent cells) in *in vitro* cultured cells.

2.4.4. Discussion on chemical, and pharmaceutical aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of

important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

The starting material to manufacture the activated pegylating reagent has been re-defined as requested. The Major Objection raised in relation to this point has been appropriately solved.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral safety.

2.5. Non-clinical aspects

2.5.1. Introduction

The toxicology program for pegunigalsidase alfa was conducted in accordance with the International Conference on Harmonisation (ICH) M3 (R2) guideline for nonclinical safety studies to support clinical trials as well as the ICH S6 (R1) guidance for biotechnology-derived products. Other relevant ICH guidelines that were followed included: ICH S3A for toxicokinetics and ICH S5 (R2) for reproductive toxicity.

The pivotal studies (chronic toxicity in two species and reproductive toxicity including fertility and early embryonic development and embryofetal development) complied with Good Laboratory Practices (GLPs).

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

The primary pharmacodynamics of pegunigalsidase alfa has been investigated *in vitro* and *in vivo* and some properties have been compared with the unmodified plant cell expressed α-GAL-A (BCL) and the two marketed commercial products: agalsidase alfa (Replagal) and agalsidase beta (Fabrazyme).

Michaelis–Menten analysis demonstrated that pegunigalsidase alfa is able to hydrolyze synthetic substrate (p-nitrophenyl-α-D-galactopyranoside) of α-Gal-A at higher rate (K_M= 2.64 mM) to agalsidase alfa (K_M=5.80 mM) and agalsidase beta (K_M=5.71 mM), indicating that the chemical modification had no negative effects on the hydrolisis kinetic parameters. Pegunigalsidase alfa also demonstrated the ability to hydrolyze the semi-natural substrate Gb3- NBD of α-Gal-A (K_M= 28.5 µM).

Covalent dimerization of α-Gal-A also resulted in significant increased stability in plasma and under acidic lysosomal-like conditions if compared with unmodified plant cell expressed α-GAL-A (BCL) and other commercial products (Replagal and Fabrazyme).

In an *in vitro* study using skin fibroblasts from two Fabry patients, pegunigalsidase alfa was shown to be internalized and localized to the lysosome, the sub-cellular target for the enzyme.

Using an animal model of Fabry disease (Fabry mice), pegunigalsidase alfa reached major target organs in the treatment of Fabry disease (skin, heart and kidney), as well as liver and spleen, and reduced the accumulation of Gb3 in all tested organs after single and repeated administrations (once every 2 weeks for a total of 6 injections) as follows: skin>heart>kidney. Pegunigalsidase alfa showed higher activity levels and longer activity

duration in the heart, kidney and spleen in comparison with BCL or Replagal (agalsidase alfa). However,the activity of pegunigalsidase alfa and Replagal (agalsidase alfa) were similar and slightly higher than that of BCL in heart and spleen and no significant differences were observed in liver and kidney. In the liver, the levels of pegunigalsidase alfa were lower than for Replagal (agalsidase alfa) and slightly higher than for the BCL. In a third Fabry mouse study, pegunigalsidase alfa produced statistically significant improvements in functional activity (nociceptive response to heat) and decreases in inflammation in the dorsal root ganglia as compared to untreated mice; meanwhile minimal effects were noted with Replagal (agalsidase alfa) or Fabrazyme (agalsidase beta).

In vivo studies demonstrated that 1 mg/kg or 2 mg/kg of pegunigalsidase alfa may provide a protective effect in mice model of Fabry disease, but different doses were not directly compared in pharmacology studies. This fact limits finding the proper dose in humans. At this time of the clinical development, it is considered acceptable to address this issue with clinical data and additional non-clinical studies are not warranted.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacology studies on pegunigalsidase alfa were performed, which is considered acceptable by the CHMP.

2.5.2.3. Safety pharmacology programme

No formal safety pharmacology studies have been conducted on pegunigalsidase alfa, but relevant information is available from the Good Laboratory Practice (GLP)-compliant 26-week repeat-dose toxicity studies performed in mice and monkeys.

Transient clinical signs such as decreased activity in monkeys and mice; dyspnoea cyanosis and abdominal position in mice and red discoloration of the face in monkeys were observed in association with the administration of pegunigalsidase alfa and were considered to be related to an allergic-type response. No effects of pegunigalsidase alfa were noted on the cardiovascular system or respiratory system in mice and monkeys.

2.5.2.4. Pharmacodynamic drug interactions

No drug interaction studies have been conducted on pegunigalsidase alfa. This was considered acceptable since on pegunigalsidase alfa is a protein and is expected to be metabolically degraded through peptide hydrolysis, thus unlikely to be candidate for cytochrome P450 mediated drug-drug interactions.

2.5.3. Pharmacokinetics

The toxicokinetics of pegunigalsidase alfa has been evaluated in mice and monkeys as well as pregnant rats and rabbits.

Methods used to detect pegunigalsidase alfa in plasma of mice, monkeys, rats and rabbits, and IgG antibodies to pegunigalsidase alfa in mice and monkey serum were adequately validated.

At the same mg/kg doses, similar C_{max} values were measured in mice, monkeys and pregnant rats treated with the same dose level, but the AUC values were higher in monkeys and rats than in mice, in the following order: monkeys>rats>mice. This was likely related to the longer mean $T_{1/2}$ (35.9, 15.3 and 7.6 hrs) and lower mean clearance (3.5, 6.13 and 13.5 mL/kg/hr) in monkeys, rats and mice, respectively. In humans, mean $T_{1/2}$ at

therapeutic doses ranged from 82.62 to 101.00 hours and C_L ranged from 1.05 to 3.41 ml/hr/kg, being more similar to values observed in monkeys. In rabbits, systemic levels after the first dose were higher compared to the other species receiving similar doses, in terms of C_{max} and AUC, which was associated to lower clearance. In mice, the increments of AUC_{0-t} and C_{max} were higher than dose-proportional between 2 and 10 mg/kg and lower than dose-proportional between 10 and 40 mg/kg. On the contrary, the increment in AUC_{0-t} in rabbits was lower than dose-proportional between 2 and 10 mg/kg and higher than dose-proportional between 10 and 20 mg/kg.

One difference noted between the species was related to the impact of repeated dosing. Whereas no change was noted for mice and rats, a decrease in AUC occurred for monkeys on Day 169 as compared to Day 1 and for rabbits on GD18. After 6 months treatment, systemic levels decreased mainly in female monkeys at 2 and 10 mg/kg, whereas ADAs were observed in 3 males at 2 mg/kg, supporting ADA development was not associated to pharmacokinetic findings. This appears to be due to a faster rate of clearance and distribution, due to higher C_L, V_z and V_{ss} values reported in groups exhibiting higher decrements in systemic levels after repeated dose, mainly females at 2 and 10 mg/kg. In case of rabbits, follow-up investigation revealed the presence of anti-pegunigalsidase alfa IgG antibodies in all animals on GD 18. The development of ADAs can be correlated to reduced plasma levels of pegunigalsidase alfa in pregnant rabbits, but ADA titers cannot be directly correlated to the degree of reduced pegunigalsidase alfa systemic levels.

No consistent gender effects were noted in PK for either species.

Analysis of the PEG moiety was not performed in human or animal's blood. Pharmacokinetic data of PEG moiety in animals after treatment with pegunigalsidase alfa were not considered necessary since the safety of PEG moiety after treatment with pegunigalsidase alfa was supported by published data from clinical experience with PEG as excipient and non-clinical and clinical data with other pegylated proteins.

Biodistribution studies confirm that pegunigalsidase alfa reached the critical tissues impacted by the disease (skin, heart and kidney) as well as liver and spleen and exposure is maintained at least for 7 days in spleen and skin and for 14 days in heart, kidney and liver. Although distribution of pegunigalsidase alfa to brain was not investigated, pharmacology studies (enzymatic activity and Gb3 levels) support that pegunigalsidase alfa does not reach this organ.

Metabolism and excretion were not studied for pegunigalsidase alfa. Pegunigalsidase alfa is expected to be enzymatically degraded into amino acids and the PEG moiety clipped off the protein. The metabolism of PEGs is described in literature (Webster et al, 2007 and Baumann A. et al, 2014). For PEG1000 and 6000, a maximum of 15% and 4% of the doses is cleared by metabolism, respectively, indicating that for the free PEG of pegunigalsidase alfa, having about 2000 Da, clearance by metabolism is a minor pathway. In any case, published data indicate that humans and animals will be exposed to similar metabolites after administration of PEG. With regard the excretion of PEG moiety, literature describes that urinary clearance is the major excretory pathway for PEGs up to 4000 Da.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No single-dose toxicity studies have been conducted on pegunigalsidase alfa.

2.5.4.2. Repeat dose toxicity

The toxicity of pegunigalsidase alfa after repeated administration was studied in CD-1 mice and Cynomolgus monkeys with dosing up to 26 weeks.

The relevance of the non-clinical species that were used to establish the safety of pegunigalsidase alfa was established via a comparison of the amino acid sequence of alpha-GAL-A in animals to humans: monkeys exhibited 98% homology versus approximately 80% homology for mice, rats and rabbits. These data establish that the safety findings in monkeys are most representative of predicting safety in humans as compared to the other species. In addition, comparison of fibroblast uptake across species revealed comparable uptake for rats, rabbits, monkeys, and humans, but the uptake and uptake-dose relationship were lower for mice. The design of toxicity studies in this species is adequate to characterize the toxicological profile of pegunigalsidase alfa.

Mortality occurred in twenty-nine mice, two of them in the control group and in two monkeys. In mice, mortality was recorded mainly following the 3rd injection and within 10-50 minutes post-dosing or 2-3 days after injection and can be considered allergic reactions, except for 2 animals in the high-dose group who died 7 and 10 days after injection. The first animal exhibited multiple foci of bacterial colonies in some organs, suggesting a systemic septic process, which can be considered as unrelated to the pegunigalsidase alfa administration. The second animal was part of the satellite group and histopathological analysis was not performed. Therefore, available data do not allow concluding whether the death of this animal might be treatment related. The cause of death of one monkey was not determined; however, this animal had mild red discoloration of multiple lung lobes that correlated microscopically with mild acute haemorrhage. The other monkey was euthanized for human reasons due to a fracture of the humerus and the inability of achieving proper stabilization of the fracture.

The main test article-related clinical signs observed in mice were a transient decrease in the spontaneous motor activity and dyspnoea. These clinical signs were initially observed in some animals following the third injection of pegunigalsidase alfa on dosing days starting at about 5 to 10 minutes post-dosing and ending at about 10 to 60 minutes post-dosing. Some animals also exhibited cyanosis and abdominal position, which in 4/13 animals occurred prior to their death. Decreased activity and red discoloration of the face or forelimbs were also observed in monkeys. Clinical signs in mice and monkeys and most mortalities in mice were considered allergic reactions to a human protein based on that they developed quickly after administration of the drug, resolved quickly, their lack of a dose-response and because DPH generally prevented progression to mortality in mice and completely resolved the signs in monkeys. This is also supported by a follow-up study in which elevated levels of platelet activating factor were observed in animals developing clinical signs. Since it may be a response to a foreign protein, these are not likely to represent a safety concern to humans.

There were no significant test article-related effects on food consumption, ophthalmoscopy, hematology, coagulation, electrocardiographic and urinalysis. Although some statistically differences were reported in treated groups compared to controls, they were of small magnitude, sporadic nature, and/or not dose-related.

The mean body weights and the mean body weight gains of male monkeys at 40 mg/kg/dose were lower than controls at the end of the study, suggesting that this dose might be close to the toxic one.

The increment of AST levels in monkeys at 2 and 40 mg/kg are probably procedure-related such as muscle injury during the handling of the monkeys, since the magnitude of AST elevation is greater than the magnitude of ALT elevation following muscle damage.

Perivascular lymphocytic infiltration was reported with higher frequency in brains, lungs, pancreas, and salivary glands of monkeys treated with 10 or 40 mg/kg of pegunigalsidase alfa and in seminal vesicles and skeletal

muscle at 40 mg/kg. Perivascular lymphocytic infiltration may be an adaptive immune response, which is an expected response in animals to a human protein and might be not clinically relevant.

In both species, microscopic changes were noted at the injection site of the control and pegunigalsidase alfa groups. These were considered to be related to the injection or infusion procedure and not test article-related as the effects occurred at a similar incidence and severity in the placebo control and pegunigalsidase alfa groups. Monkeys exhibited minimal to mild vascular degeneration/necrosis, minimal to moderate haemorrhage, minimal to mild acute inflammation, and minimal erosion/ulcer. All changes in the mice were minimal in severity and included focal blood vessel necrosis, multifocal perivascular haemorrhage, and multifocal perivascular inflammation.

Microscopic adverse effects observed in the kidneys consisted of increased incidence and/or mean severity (minimal to mild) of multifocal nephropathy and minimal to mild interstitial lymphocytic infiltration in comparison to control animals. These microscopic adverse effects were not seen in the kidneys of the low and mid-dose animals, but these doses do not provide enough safety margin (<0.5) to discard the renal toxicity in humans.

With regard to liver toxicity, hepatocytic vacuolation consistent with fatty change was observed in one male mouse treated with 40 mg/kg, the incidence hepatocyte necrosis was higher in treated male and female mice. Hepatocyte necrosis was only observed in the interim group in males, while in females, it was observed in the interim, terminal and recovery groups. Moreover, minimal to mild Kupffer cell hypertrophy was found in monkeys.

The relevance of toxicity studies with pegunigalsidase alfa to assess the safety of free PEG moiety is limited by the lack of exposure data to free PEG in humans and animals after administration of the enzyme. Therefore, the safety of free PEG is supported by published data from clinical experience with PEG as excipient and non-clinical and clinical data with other pegylated proteins. PEG molecules are generally regarded as inert and they have no specific receptor or other target proteins in tissues. Literature also suggests that the primary toxicological effects of PEG are cellular vacuolation and hypersensitivity reactions related to anti-PEG antibody formation. Hepatocytic vacuolation was observed in one male mouse treated with pegunigalsidase, which was associated to mixed cell infiltration.

2.5.4.3. Genotoxicity

No genetic toxicity studies have been conducted on pegunigalsidase alfa, which is acceptable due to the nature of the product.

PEG moieties of 0.2–60 kDa have been assessed for the mutagenic and chromosome aberration potential in other pegylated proteins, and no evidence of genotoxic potential were observed.

2.5.4.4. Carcinogenicity

Pegunigalsidase alfa was not evaluated for carcinogenic potential.

The α-GAL-A enzyme is naturally occurring in the body, so the presence of alpha galactosidase *per se* is not associated with any carcinogenic risk. Since pegunigalsidase alfa will be administered to patients with deficiency of α-GAL-A, the risk of carcinogenicity due to exposure to pegunigalsidase alfa levels that exceed enzyme levels biologically present in the body is low and the risk arising from of the manufacturing process (impurity, reagents, solvents…) is controlled by DS and DP specifications.

Published carcinogenicity studies with PEG did not reveal carcinogenic potential, although they did not follow the ICH requirement (GLP compliance, duration, number of dose tested…). Moreover, PEGylated proteins have been used in patients for many years and have not shown any indication of a carcinogenic potential. Therefore, there is limited concern regarding the carcinogenic potential of free PEG after pegunigalsidase alfa treatment.

2.5.4.5. Reproductive and developmental toxicity

In Sprague Dawley rats, treatment with pegunigalsidase alfa before and during mating and through implantation did not have any adverse effects on reproduction and fertility parameters up to the highest dose tested, 40 mg/kg. Fertility studies showed that IV administration of pegunigalsidase alfa did not affect mating or fertility.

The potential of pegunigalsidase alfa to induce developmental toxicity after maternal exposure during organogenesis was evaluated in rats and rabbits following intravenous administration. In rats, dosing up to 40 mg/kg/dose produced no clinical signs, mortality or adverse effects on litter parameters (including total number of fetuses, number of live fetuses, mean litter size, mean fetal weights of males and female fetuses and sex ratio). In comparison, the embryofetal development study in rabbits resulted in maternal toxicity (mortality, abortions, and decreased weight gain) at 10 and 20 mg/kg/dose, which resulted in secondary decreases in fetal body weights. In both species, a statistically significant increase in the percent incidence of skeletal alterations was observed in the test article groups as compared to the vehicle control group. Most alterations were considered no biological significant because their incidences were within historical control data or the lack of dose-response.

No prenatal and postnatal developmental studies have been conducted on pegunigalsidase alfa. PK assessment in fetus and suckling pups indicated that they are exposed to pegunigalsidase alfa through placenta and milk. Low systemic exposure in fetus (between 0.005 and 0.025% of dams' systemic exposure) and suckling pups (maximum 0.014% compared to mother's systemic exposure) were reported following repeated treatment with pegunigalsidase alfa.

2.5.4.6. Toxicokinetic data

Toxicokinetic data have been collected from pharmacokinetics, repeated dose toxicity and reproductive and development toxicity studies and are discussed in 2.5.3.

2.5.4.7. Local Tolerance

Local tolerance was assessed as part of repeat dose toxicity studies and are discussed in 2.5.4.2.

2.5.4.8. Other toxicity studies

The presence of antibody anti-pegunigalsidase alfa in mice and monkeys did not impact the toxicokinetic findings. On the contrary, anti-pegunigalsidase alfa IgG antibodies in rabbits can be correlated to reduced plasma levels of pegunigalsidase alfa.

2.5.5. Ecotoxicity/environmental risk assessment

Pegunigalsidase alfa is an enzyme consisting of naturally occurring amino acids linked to a PEG moiety. By their nature, it is unlikely to result in significant risk to the environment.

According to the referred database (ECHA) the PEG moiety does not result in a significant risk to the environment because its rapid biodegradation in the environment. PEGs do not fulfil the PBT criterion either. The patient population for the proposed enzyme replacement therapy is very limited indeed, thus it is acceptable that the amount of PEG released into the environment is low compared to amount of PEG released into the environment upon other pegylated compounds (cosmetics and already approved drug products).

2.5.6. Discussion on non-clinical aspects

The pharmacodynamic and pharmacokinetic effects of pegunigalsidase alfa have been sufficiently characterised through non clinical studies using both in vitro and in vivo settings.

Comparative data versus unmodified plant cell expressed α-GAL-A (BCL) and Replagal (agalsidase alfa) using animal disease model (Fabry mice) showed higher activity levels and longer activity duration in the heart, kidney and spleen with pegunigalsidase alfa treatment. However, the increment of distribution to organs was not associated with a significantly increased efficacy. Furthermore, statistically significant improvements in functional activity (nociceptive response to heat) and decreases in inflammation in the dorsal root ganglia were observed as compared to untreated mice, whereas minimal effects were noted with Replagal (agalsidase alfa) or Fabrazyme (agalsidase beta). The CHMP noted that inflammation can be associated with thermal insensitivity, amongst other responses.

The causes of the different PK profile of pegunigalsidase alfa in pregnant rabbits are unknown, but due to clinical experience with pegunigalsidase and since PK data in humans are available, further non-clinical follow up is not warranted. Although the mean Cmax and AUC0-t values increased with increasing dose in all non-clinical species, the pharmacokinetic was not linear in mice and rabbits. Plasma levels in fetuses and pups are significantly lower than in mothers but the available pharmacology studies do not allow to determine the minimum pharmacological active dose and due to drug-sensitivity, it may be different at various development stages. Other potential non-pharmacological adverse effects on developmental organs (e.g. cell vacuolation) during late pregnancy and lactation cannot be excluded. Section 5.3 of the SmPC describes the lack of prenatal and postnatal developmental studies and its potential clinical consequences: risks for fetus and pups during the late pregnancy and lactation are unknown.

The main target organs of pegunigalsidase alfa toxicity were kidneys (in mice) and liver (in mice and monkeys).

The reported increased incidence and/or mean severity of multifocal nephropathy and interstitial lymphocytic infiltration in the kidneys are described in section 5.3 of the SmPC. Furthermore, glomerulonephritis membranoproliferative, chronic kidney disease and proteinuria have been observed in patients after treatment with pegunigalsidase alfa and have been included as adverse drug reactions (ADRs) in section 4.8 of SmPC and thus, no further non-clinical follow up is required.

Similar to kidney findings, the risk of hepatotoxicity for humans cannot be excluded based on exposure data, but regarding liver, no adverse effects have been reported in humans. Cellular vacuolation is a known risk of PEGylated proteins and Kupffer cell hypertrophy may be an immune response to a foreign protein. Altogether, these data support that liver may be a target organ of pegunigalsidase alfa and microscopic findings in livers of mice and monkeys are described in section 5.3 of the SmPC.

Despite the low incidence of the hepatocytic vacuolation (1/19), this finding is described in section 5.3 of the SmPC because it is a known risk of PEGylated proteins, there is no safety margin for humans and the long-term consequences of PEG accumulation and deposition in the body is still not fully characterised. With regard

anti-PEG antibody response, immunogenicity analyses in non-clinical animal studies are not relevant in terms of predicting potential immunogenicity in humans, thus this potential risk are further discussed in section 2.6.

The lack of genotoxicity studies with pegunigalsidase alfa is acceptable due to the nature of the product. Since pegunigalsidase alfa is an enzyme replacement treatment, the risk of carcinogenicity is low.

Pegunigalsidase alfa is unlikely to result in significant risk to the environment.

2.5.7. Conclusion on the non-clinical aspects

Overall, the non-clinical aspects of pegunigalsidase alfa have been adequately documented and meet the requirements to support this application.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 3: Completed and ongoing clinical studies with PRX-102 in this MAA

Type of study	Identifier	Objectives	Treatment	Participants
Phase 1/2 Completed	PB-102-F01 PB-102-F02	Dose finding, safety, PK, exploratory efficacy	PRX-102 0.2, 1.0, or 2.0 mg/kg IV Q2W for 12 weeks in PB102-F01 and for additional 9 months in PB102-F02 Duration: 12 months	n=18 [a] 0.2 mg/kg: n=6 1.0 mg/kg n=8 2.0 mg/kg: n=4 Adult male / female patients with Fabry disease previously untreated [b]
Phase 1/2 Completed	PB-102-F03	Safety, exploratory efficacy OLE study following studies PB-102-F01 and PB-102-F02	PRX-102 1.0 mg/kg IV Q2W [d] Duration: Up to 60 months	n=15 Adult male / female patients with Fabry disease previously treated with PRX-102

Phase 3 Ongoing * 12 months interim analysis completed	PB-102-F20 BALANCE	Efficacy, safety, PK	PRX-102 1.0 mg/kg, IV Q2W Active control: agalsidase beta (Fabrazyme) 1.0 mg/kg, IV Q2W stratified by urine protein/creatinine ratio Duration: 24 months	n=77 [a] PRX102 n=52 Fabrazyme n=25 exposed Adult male / female patients with Fabry disease, previously treated with Fabrazyme, Limit of 50% female patients
Phase 3 Completed	PB-102-F30 BRIDGE	Safety, exploratory efficacy	PRX-102 1.0 mg/kg IV Q2W switch of agalsidase alfa to PRX-102 1 after a 3 month evaluation period while on agalsidase alfa (Replagal)[f] Duration: 12 months	n=22 Adult male / female patients with Fabry disease, previously treated with agalsidase alfa. Limit of 25% female patients
Phase 3 Completed	PB-102-F50 BRIGHT	Safety, exploratory efficacy, PK	PRX-102 2.0 mg/kg IV Q4W switch of agalsidase alfa (Replagal) or beta (Fabrazyme) [g] Duration: 12 months	n=30 Adult male / female patients with Fabry disease previously treated with either agalsidase alfa or beta. Limit of 20% female patients.
Phase 3 Ongoing	PB-102-F51	Long-term safety, exploratory efficacy OLE study of Study PB-102-F50	PRX-102 2.0 mg/kg Q4W Duration: Up to 48 months	n=29 Adult male / female patients continuing from PB-102-F50
Phase 3 ongoing	PB-102-F60 BRILLIANCE	Long-term safety, exploratory efficacy OLE study of PB-102-F03, PB 102-F20 and PB-102-F30	PRX-102 1.0 mg/kg Q2W Duration: Up to 60 months	n=69 n=10 from F03, n=18 from F30 n=41 from F20 Adult male / female patients with Fabry disease

EOW: every other week; *NA*: not applicable; *OLE*: open-label extension; *Q2W*: every 2 weeks; *Q4W*: every 4 weeks
[a] *PB-102-F01*: Nineteen (19) patients enrolled, 1 withdrew consent prior to administration of study drug. *PB-102-F20*: Seventy-eight (78) patients enrolled, 1 withdrew consent prior to administration of study drug
[b] *Naïve*: patients not previously exposed to an ERT or off-treatment for at least 6 months
[c] *Studies PB-102-F01 / PB-102-F02* had two separate protocols but were analyzed in a single CSR
[d] After gradual dose adjustment to 1.0 mg/kg EOW for additional 36-60 months in PB-102-F03
[e] On Fabrazyme for at least 1 year and on a stable dose (≥80% labelled dose/kg) for at least 6 months
[f] On Replagal at least 2 years and on a stable dose (>80% labelled dose/kg) for at least 6 months
[g] On Replagal or Fabrazyme for at least 3 years and on a stable dose (>80% labelled dose/kg) for at least 6 months
[h] 26 patients coming from pegunigalsidase alfa arm and 15 from Fabrazyme arm of study PB-102-F20

*ongoing at time of initial submission, final clinical study report submitted during the procedure

2.6.2. Clinical pharmacology

The clinical pharmacological program for pegunigalsidase alfa (PRX-102) encompasses eight completed and ongoing clinical Phase 1/2 and Phase 3 studies. See Table 3.

2.6.2.1. Pharmacokinetics

Pegunigalsidase alfa in plasma was detected via ELISA assays. Regarding immunogenicity, different methods were used.

PK data were analysed using non-compartmental analysis (NCA) and population PK modelling. Population PK modelling and PK/PD analyses were performed using pooled data from studies PB-102-F01, PB-102-F02, PB-102-F20, and PB-102-F50 (Reports ICX-B152, ICX-B160, ICX-B173 MSAR1, and ICX-B173 MSAR2). For population PK modelling, the nonlinear-mixed effects modelling approach with NONMEM software was used. No particular statistical analysis outside of population PK was performed. Due to the inadequacy of the original population PK and PK/PD (or [dose]-exposure-response) model, an updated model using available data from adults was requested by the CHMP in order to support an adequate characterisation, description and prediction the PK (and PD) of pegunigalsidase alfa in this population. However the most relevant model as presented by the applicant (ICX-B173 MSAR2) during the procedure still had misspecifications in structure and was not considered reliable and predictable. For this reason, only graphical representation is provided below regarding potential ADA effect on PK/PD of pegunigalsidase alfa and relationship between plasma concentration and response, in addition to data based on NCA.

Absorption

Following IV infusion, pegunigalsidase alfa was immediately bioavailable.

Distribution

Pegunigalsidase alfa is not expected to bind to plasma proteins.

Elimination

The excretion of PRX-102 has not been evaluated. This is acceptable due to the nature of the product. Furthermore, the molecular weight of pegunigalsidase alfa is ~116 KDa, which is twice the cut-off value for glomerular filtration, thus excluding filtration and/or proteolytic degradation in kidneys. Based on NCA, $t_{1/2}$ ranges from 53 to 134 h (minimum 53.4 h in study PB-102-F01 and Study PB-102-F02, and maximum 133.7 h in PB-102-F50).

Dose proportionality and time dependencies

For patients who received 1 and 2 mg/kg Elfabrio, there were increases in mean $t_{1/2}$ and $AUC_{0-\infty}$ with increasing duration of treatment and corresponding decreases in Cl and V_z, suggesting a saturated clearance. In the same studies, a definite time and dose effect as Cl and Vz decrease with the numbers of doses received could be seen, and that effect was even more marked with highest doses.

Special populations

The effect of special populations (i.e. gender, race, ethnicity, and age) or other covariates (weight) on the PK behaviour of pegunigalsidase alfa is not fully clear. In particular, whereas study PB-102-F20 may show a

gender effect, with a small number of subjects and possibly an ADA-effect covariate, study PB-102-F50 did not show any covariate effect on PK/PD of pegunigalsidase alfa in the adult population.

No studies have been carried out in subjects with renal or hepatic impaired function.

The final population PK model, as presented by the applicant (report ICX-B173 MSAR2), is a 3-compartment model with zero-order absorption, first-order elimination and interindividual variability on CL, V1, Q3, and V3. A combined error model was used stratified by Study PB-102-F01/ PB-102-F02 versus the other two studies. Inter-occasion variability on bioavailability was implemented. Covariates were identified for IgG titer on CL and V1 (Emax models), and study PB-102-F01/PB-102-F02 on CL, V1, Q3, and V3.

ADA positive patients seem to have remarkably lower exposure compared to ADA negative patients (Figure 3, Figure 4, and Figure 5). Thus ADA formation might affect the therapeutic effect.

Figure 3: Pegunigalsidase alfa plasma concentrations (overlaid with smooth red line) at each PK visit stratified by ADA status at baseline for patients from Study PB-102-F01/F02, report ICX-B173 MSAR2

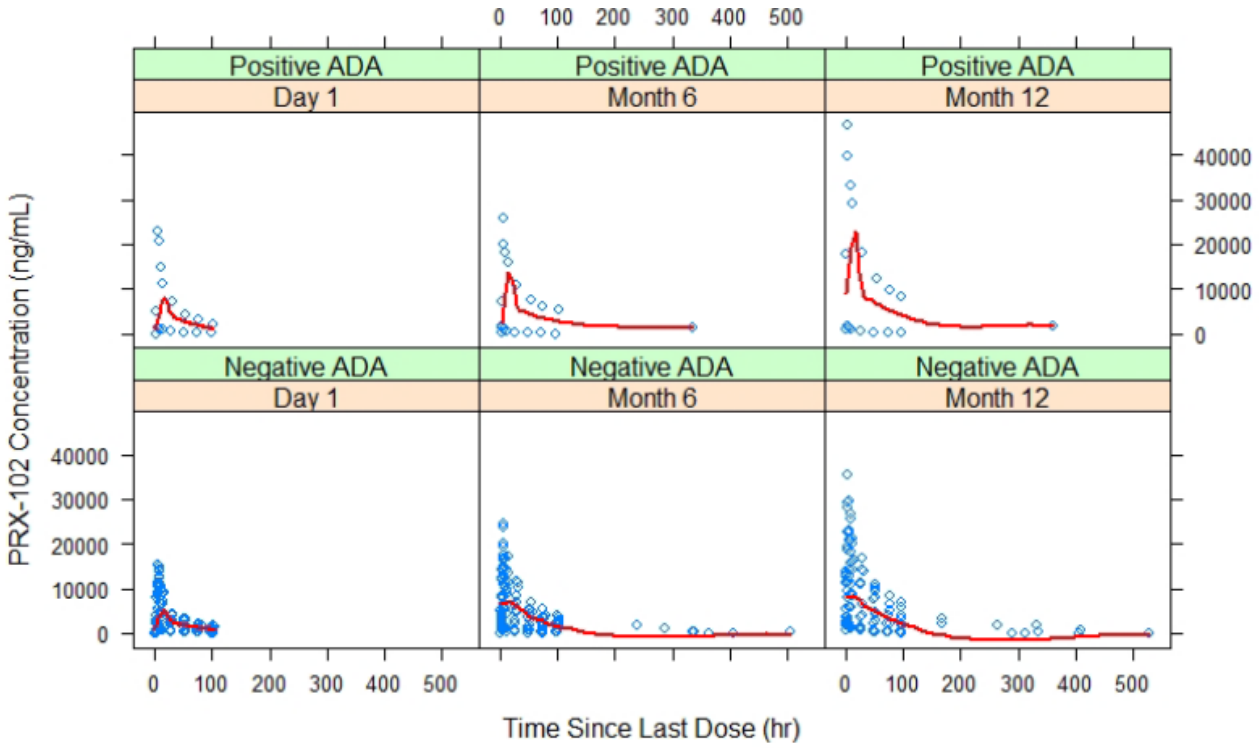


Figure 4: Pegunigalsidase alfa plasma concentrations (overlaid with smooth red line) at each PK visit stratified by ADA status at baseline for patients from Study PB-102-F20, report ICX-B173 MSAR2

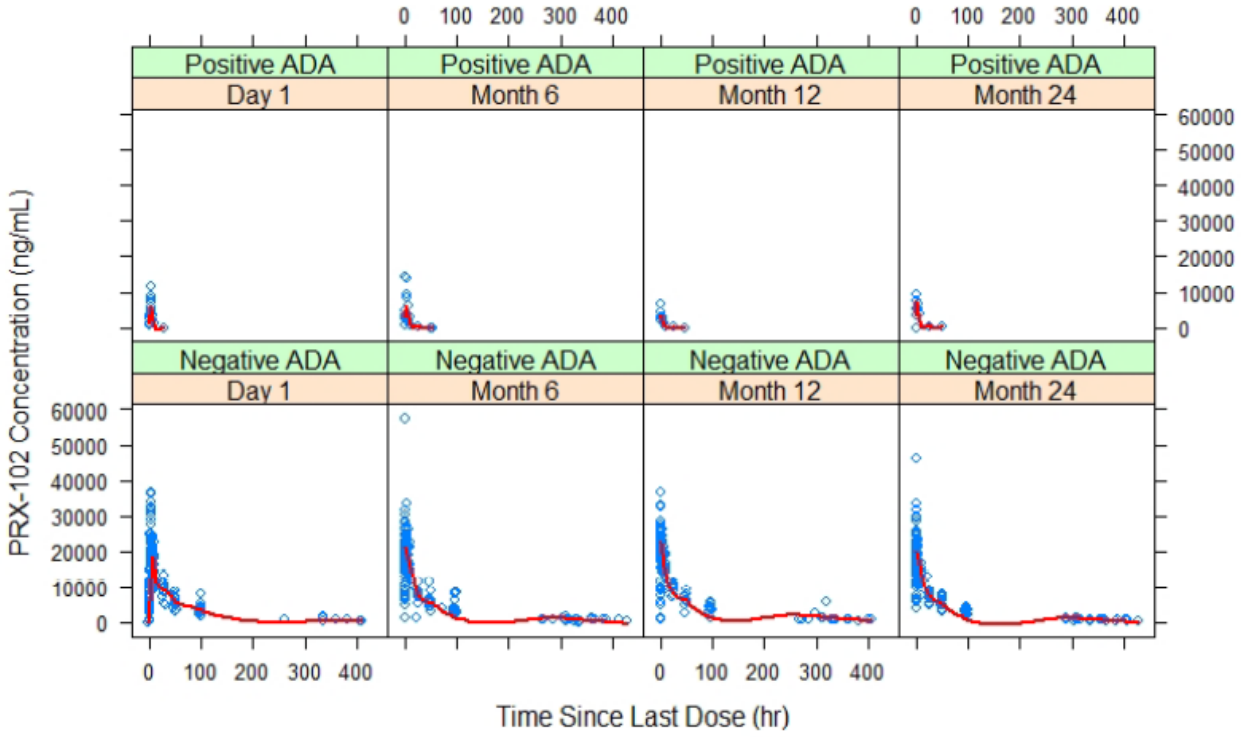
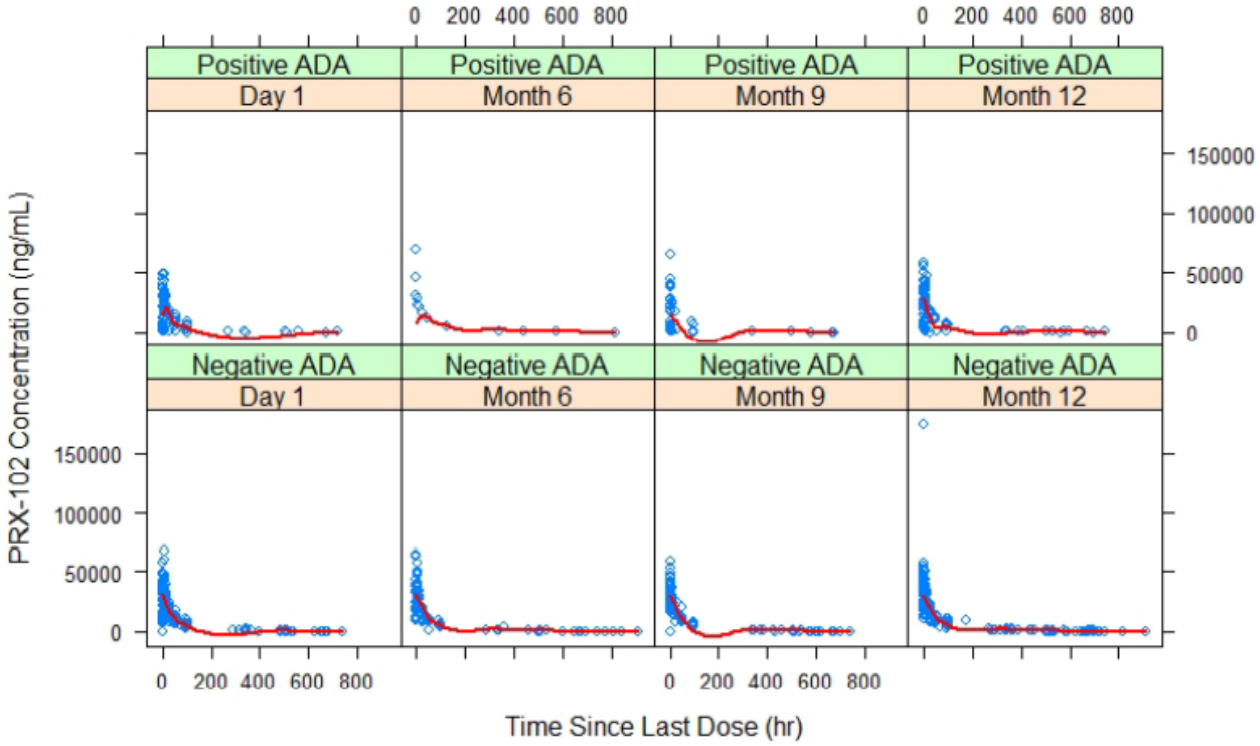


Figure 5: Pegunigalsidase alfa plasma concentrations (overlaid with smooth red line) at each PK visit stratified by ADA status at baseline for patients from Study PB-102-F50, report ICX-B173 MSAR2



Pharmacokinetic interaction studies

No interaction studies have been performed. Because it is a recombinant human protein, pegunigalsidase alfa is an unlikely candidate for cytochrome P450 mediated drug-drug interactions.

Pharmacokinetics using human biomaterials

See above.

2.6.2.2. Pharmacodynamics

Mechanism of action

The active substance of Elfabrio is pegunigalsidase alfa. Pegunigalsidase alfa is a pegylated recombinant form of human α-galactosidase-A. The amino acid sequence of the recombinant form is similar to the naturally occurring human enzyme.

Pegunigalsidase alfa supplements or replaces α-galactosidase-A, the enzyme that catalyses the hydrolysis of the terminal α-galactosyl moieties of oligosaccharides and polysaccharides in the lysosome, reducing the amount of accumulation of globotriaosylceramide (Gb3) and globotriaosylsphingosine (Lyso-Gb3).

Primary and Secondary pharmacology

Renal Gb3 inclusion bodies and plasma globotriaosylsphingosine (Lyso-Gb3) concentrations are the main PD markers that were followed as part of PB-102-F01/02 and PB-102-F03.

In study PB-102-F01, kidney biopsies of peritubular capillaries (PTCs) were analysed for Gb3 inclusions using the quantitative Barisoni Lipid Inclusion Scoring System (BLISS) methodology. Overall, 11 out of 14 (78.6%) patients with available biopsies had substantial reduction (≥50%) in their BLISS score following 6 months of treatment with pegunigalsidase alfa and a reduction of ≥20% was achieved by 12 out of 14 (85.7%). At any dose, Gb3 inclusions were reduced from a mean (SE) score of 4.3 (0.9) at baseline to 0.8 (0.2) after 6 months of pegunigalsidase alfa treatment (mean [SE] reduction in score of 67.8% [8.9%], n=13).

Data on plasma Lyso-Gb3 in treatment-naïve patients, males and females, respectively were considered the most informative Fabry disease-specific biomarker and are presented in Error! Reference source not found. and Error! Reference source not found.. Plasma Lyso-Gb3 was evaluated before dosing initiation, every 3 months for the first 36 months (including the 12 months from PB-102-F01/F02), and every 6 months after 36 months of treatment.

For treatment-naïve patients with Fabry disease (n=16), mean (±standard error [SE]) plasma Lyso-Gb3 concentration at baseline was higher in males (Treatment Group I: 134.2±47.2 ng/mL; II: 100.6±39.4 ng/mL; and III: 61.8 ng/mL) than in females (13.4±5.9 ng/mL; 10.6±3.8 ng/mL; and 6.4±2.3 ng/mL) in all cohorts, consistent with the expectation of the disease state.

Analysis of the overall population, including patients who completed 12 months of treatment with pegunigalsidase alfa (n=16), indicates that Lyso-Gb3 concentration decreased significantly in response to the treatment (p=0.010), from a mean of 66.7±19.5 to 22.6±5.1 ng/mL. All 16 patients showed a reduction in plasma Lyso-Gb3 concentration from Baseline, ranging from -0.4 to -203.4 ng/mL.

In all 3 treatment groups, a greater mean reduction from Baseline was observed in males (Treatment Group I: -61.8% mean reduction, n=4; II: -67.7% mean reduction, n=4; and III: -50.2% mean reduction, n=1) than in females (Treatment Group I: -6.6% mean reduction, n=2; II: -44.5% mean reduction, n=2; and III: -37.2% mean reduction, n=3.

A reduction in plasma Lyso-Gb3 concentration was observed across all doses; however, the most pronounced reduction (mean -51.6 ng/mL; -59.9%) was observed at 1.0 mg/kg (n=6). A responder analysis indicated that the majority (56.3%) of patients had a greater than 50% reduction and 12.5% had a greater than 75% reduction in plasma Lyso-Gb3 levels after 12 months of treatment.

Figure 6 Change in Lyso-Gb3 Over Time in Males (PB-102-F01/F02)

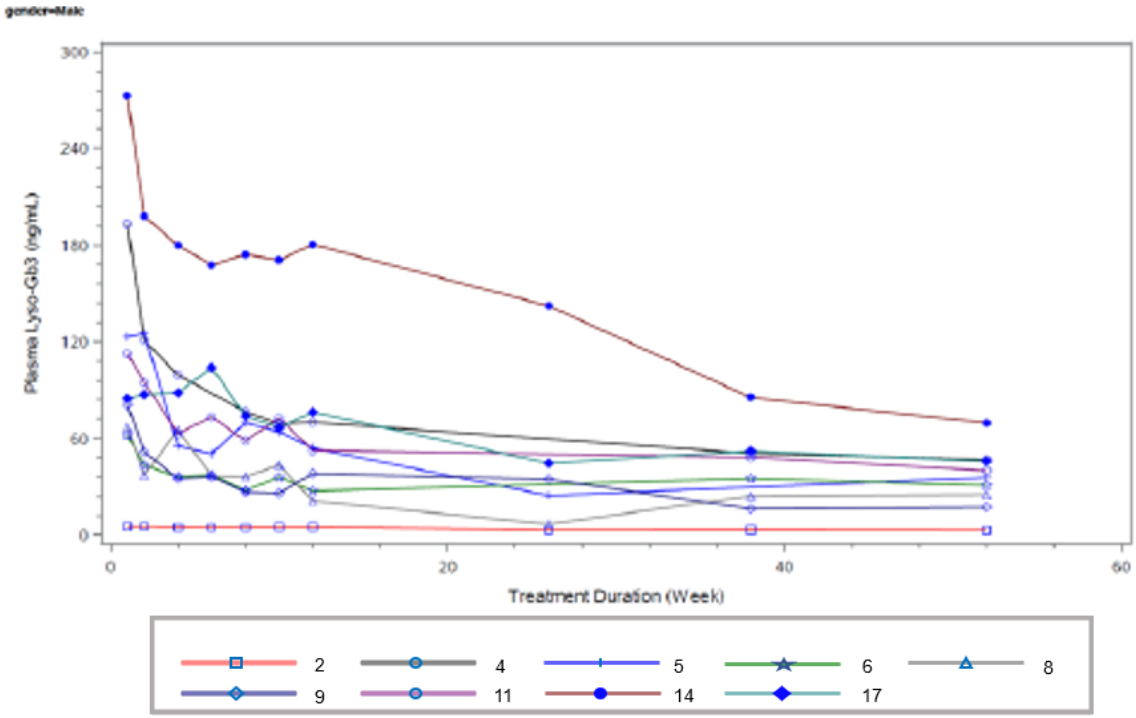
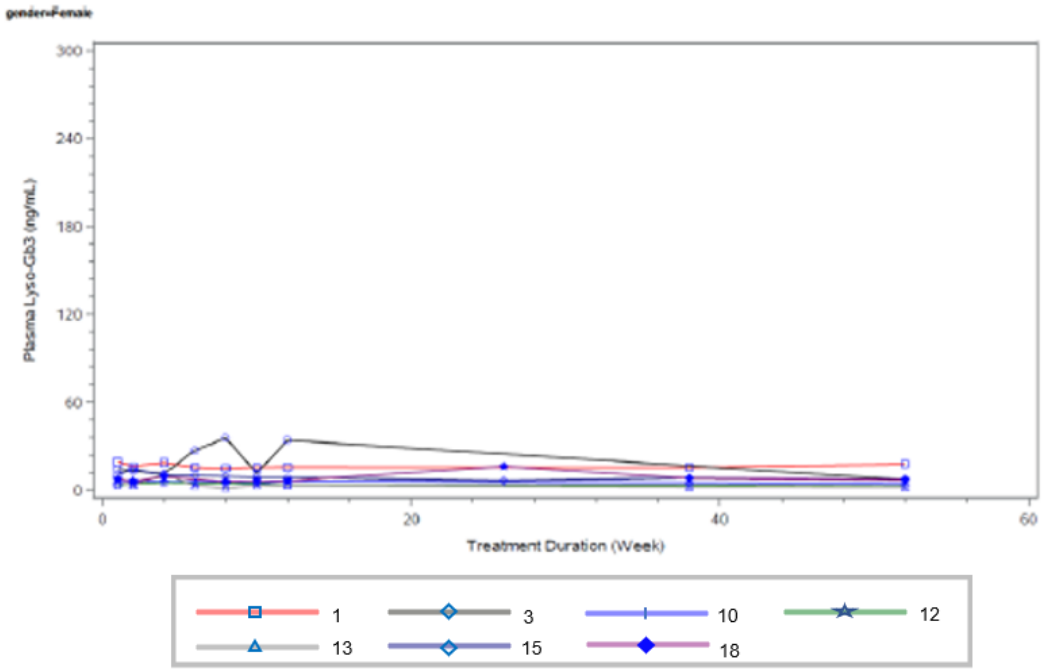


Figure 7 Change in Lyso-Gb3 Over Time in Females (PB-102-F01/F02)



In the final population PK model, as presented by the applicant (report ICX-B173 MSAR2), an exploratory PK/PD analysis for pegunigalsidase plasma concentrations and plasma Lyso-Gb3. Observed plasma Lyso-Gb3 concentrations over time for studies PB-102-F01, PB-102-F02, PB-102-F50 and PB-102-F20 are presented in Figure 8 and Figure 9. Female patients had little or no elevated levels of Lyso-Gb3 at baseline and no reduction in Lyso-Gb3 with increase in exposure of pegunigalsidase alfa (Figure 8). Concentration – effect-relationships can be observed only in treatment naïve male patients. For male patients switching from prior treatments (Studies PB-102-F20 and PB-102-F50) to pegunigalsidase alfa, data suggest the Lyso-Gb3 levels remain stable and are not correlated with exposures pegunigalsidase alfa. Relative changes in Lyso-Gb3 in Studies PB-102-F20 and PB-102-F50 were not considered to be clinically meaningful.

Figure 8: Plasma Lyso-Gb3 concentrations (grey line: observed data, red line: smooth) by study day and stratified by study and sex for patients that were part of the population PK analysis report ICX-B173 MSAR2

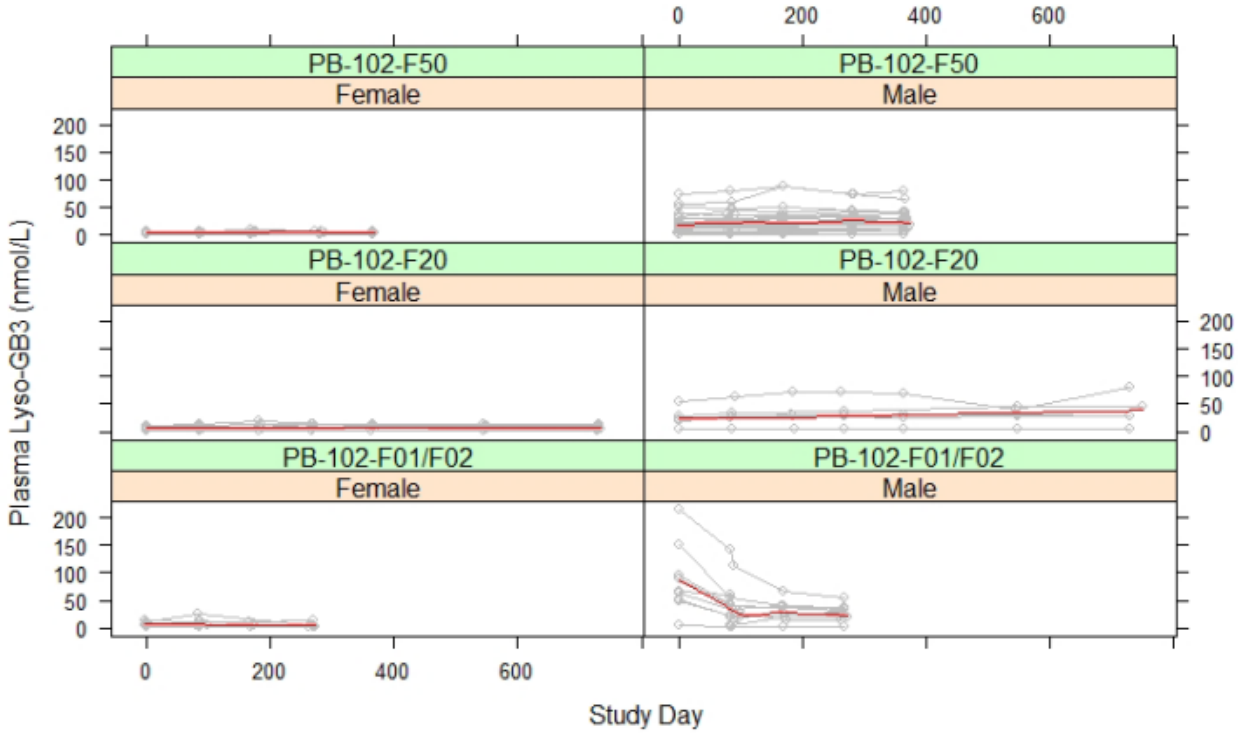
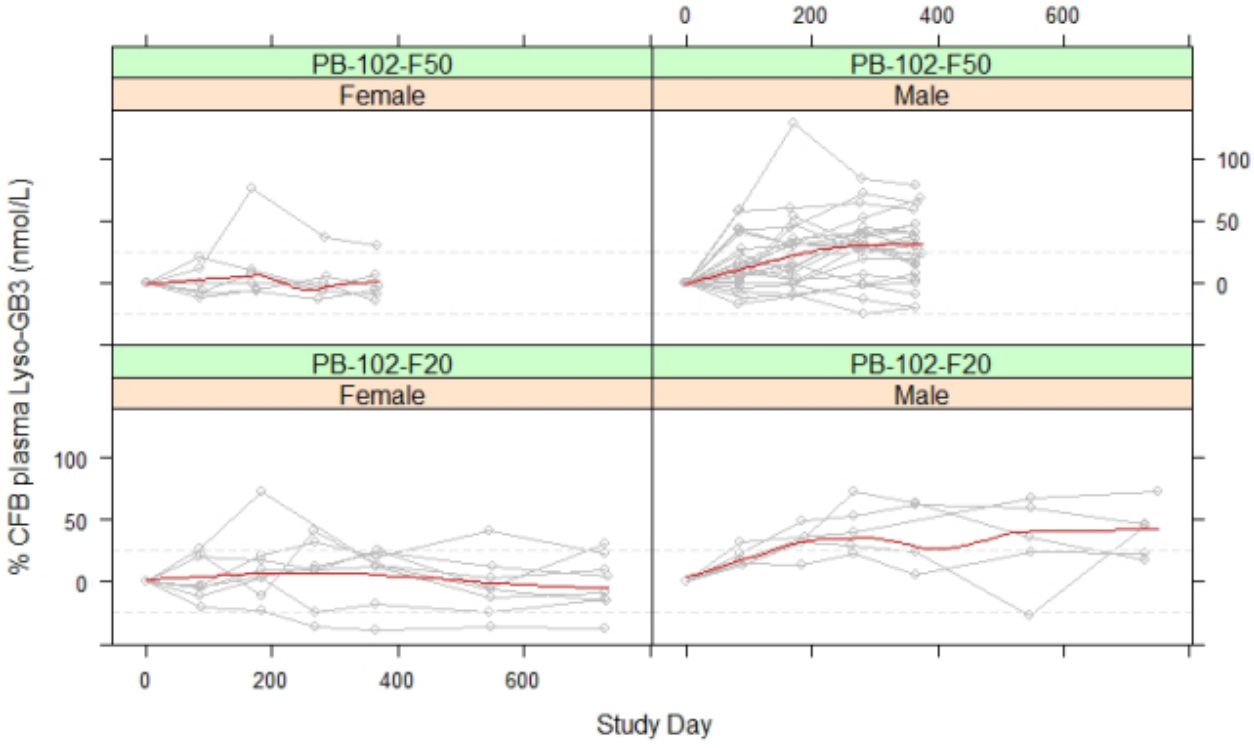


Figure 9: Percent change from baseline in plasma Lyso-Gb3 levels by study day and stratified by study and sex for patients from Studies PB-102-F20 and PB-102-F50 that were part of the population PK analysis in report ICX-B173 MSAR2



2.6.3. Discussion on clinical pharmacology

The pharmacokinetic profile of pegunigalsidase alfa has been sufficiently characterised. Nevertheless, in studies PB-102-F01 and PB-102-F02, dose proportionality was not demonstrated, and this can be reassessed with further data once available.

The impact of the quality differences on the resulting DS batches used during pre-clinical studies, clinical studies and for commercial manufacture can be neglected (see quality). Thus, the use of different pegylation reagents is deemed not significant on the evaluation of the pharmacokinetic profile of pegunigalsidase and the bioanalytical data (both pegunigalsidase and ADA measurements).

At initial submission, the proposed recommended dose of PRX-102 is 1 mg/kg administered once every two weeks (Q2W or EOW) by intravenous (IV) infusion or 2 mg/kg administered once every four weeks (Q4W or E4W) by IV infusion. The choice between the two posology options was to be selected based on the clinical judgement, patient compliance and response to treatment.

Although analysis of PK data from the 2 mg/kg pegunigalsidase alfa Q4W were provided during the procedure and were considered comparable to Q2W, such an alternative regimen was not considered acceptable by the CHMP based on the presented evidence on efficacy (see section 2.6.6). In addition, due to several deficiencies, the final population PK model, as presented by the applicant, could not be used to guide dosing strategies.

Thus, the CHMP recommended a posology of 1 mg/kg Q2W as investigated in the main study and in line with the dose finding data (see section 2.6.6). The CHMP noted that although elimination half-life of pegunigalsidase alfa seems much longer compared to other approved ERTs in the same indication, the dosing frequency remains the same (Q2W) and as such, a claim for improvement of the PK properties due to the pegylation cannot be made. Furthermore, the relationship between the elimination half-life and doses remains unclear in the absence of comparative data from other approved ERTs of the same indication.

The PK profile of pegunigalsidase alfa is not expected to be impacted by hepatic or renal impairment. No dose adjustment is not recommended in these populations.

Since the final PK/PD modelling, as presented by the applicant, is considered inadequate by the CHMP, relevant SmPC information is based on data from NCA only.

Data from the main PD markers, renal Gb3 inclusion bodies and plasma globotriaosylsphingosine (Lyso-Gb3 concentrations) are supportive of the efficacy of pegunigalsidase alfa since such changes are not anticipated without effective ERT. Specifically:

- Gb3 inclusions were reduced from a mean (SE) score of 4.3 (0.9) at baseline to 0.8 (0.2) after 6 months of pegunigalsidase alfa treatment (mean [SE] reduction in score of 67.8% [8.9%].
- A reduction in plasma Lyso-Gb3 concentration was observed across all doses; the most pronounced reduction (mean -51.6 ng/mL; -59.9%) was observed at 1.0 mg/kg (n=6). A responder analysis indicated that the majority (56.3%) of patients had a greater than 50% reduction and 12.5% had a greater than 75% reduction in plasma Lyso-Gb3 levels after 12 months of treatment. A clear trend between pegunigalsidase alfa exposure and change in plasma Lyso-Gb3 could not be observed.

Based on graphical investigations (see Figure 3, Figure 4, and Figure 5), ADA positive patients appeared to have lower therapeutic effect. More pronounced differences in PK concentrations and parameters correlated with patient ADA titers and thus it is clear that the immune response against PRX-102 is an important predictor of the plasma levels. However, the shape of this relationship is not clear since many patients who

were switched from the standard therapy to PRX-102, already had neutralizing antibodies to PRX-102 at Day 1. The PRX-102 levels in these patients were much lower, sometimes zero. PRX-102 is dosed by body weight but the dependence of PRX-102 levels on body weight is not clear, in the absence of an adequate PK/PD modelling. These uncertainties can be reassessed with further data once available.

2.6.4. Conclusions on clinical pharmacology

Overall, the pharmacological profile of pegunigalsidase alfa in human studies has been adequately documented and meet the requirements to support this application.

2.6.5. Clinical efficacy

2.6.5.1. Dose response study(ies)

PB-102-F01 was an open-label, dose ranging study to evaluate the safety, tolerability, pharmacokinetics, immunogenicity and exploratory efficacy parameters of pegunigalsidase alfa (PRX-102) in adult (>18 years of age) Fabry patients. Patients were enrolled into one of three pegunigalsidase alfa (PRX-102) treatment groups (0.2, 1.0 or 2.0 mg/kg; up to 6-8 patients per group) and received intravenous infusions every 2 weeks for 12 weeks (total of 7 infusions). Patients who enrolled into the extension study PB-102-F02 were to continue to receive the same dose of pegunigalsidase alfa (PRX-102) they received in study PB-102-F01 every 2 weeks for 38 weeks (9 months).

Patients who completed 12 months treatment of pegunigalsidase alfa (PRX-102) were eligible to enter PB-102-F03 for an additional 24-month treatment period which was further amended to a 60-month study duration. The aim of BP 102-F03 was to evaluate the ongoing safety, tolerability and efficacy parameters.

Inclusion in PB-102-F01 was open to symptomatic adult Fabry patients (≥18 years, males and females) who had never received ERT or had not received ERT in the past 6 months and had a negative anti-pegunigalsidase alfa (PRX-102) antibody test (ERT-naïve). Males were required to have plasma and/or leukocyte α-GAL-A activity less than lower limit of normal in plasma and/or leukocytes, while females were required to have historical genetic test results consistent with Fabry mutations. All patients had to have an eGFR ≥ 60 mL/min/1.73 m^2 and globotriaosylceramide (Gb3) concentration in urine > 1.5 times upper limit of normal (ULN). Patients were excluded if they had severe kidney or cardiac disease or if ACEi or ARB therapy was initiated, or the dose changed in the 4 weeks before screening.

Three doses of pegunigalsidase alfa (0.2, 1.0, 2.0 mg/kg) were administered sequentially with an intention of a minimum of 2 females and 4 males to be enrolled per dose group before the subsequent dose group was enrolled. The selection of the two lower doses of pegunigalsidase alfa (PRX-102) in this study was to provide dosing equivalent to the referenced regimens of Fabrazyme (0.2 mg/kg) and Replagal (1.0 mg/kg).

Study PB-102-F01/ PB-102-F02

Up to 24 adult patients were planned to be enrolled into study PB-102-F01. Nineteen (19) patients were eligible for enrollment. Six (6) patients were enrolled in the 0.2 mg/kg treatment group, 9 in the 1.0 mg/kg and 4 in the 2.0 mg/kg treatment groups. Two (2) patients in the 1.0 mg/kg treatment group discontinued from the study, one experienced a hypersensitivity reaction and one was non-compliant.

Sixteen (16) patients completed study PB-102-F01 (3 months) and all 16 patients enrolled into the extension study PB-102-F02. All sixteen (16) patients completed study PB-102-F02. Fifteen (15) patients (one patient in

the 1.0 mg/kg treatment group declined further participation) were enrolled into Extension study PB-102-F03 all the patients were to receive 1.0 mg/kg.

Demographics characteristics are presented in Table 4.

Table 4 Demographics - Safety Population

		PRX-102 0.2 mg/kg N = 6	PRX-102 1.0 mg/kg N = 8	PRX-102 2.0 mg/kg N = 4
AGE (yrs)	N	6	8	4
	MEAN (SE)	30.0 (4.4)	33.5 (4.1)	40.0 (8.2)
	SD	10.8	11.7	16.5
	MEDIAN	26.0	30.0	43.0
	RANGE	21 to 50	17 to 52	20 to 54
AGE AT TREATMENT (yrs)	N	6	8	4
	MEAN (SE)	30.0 (4.4)	33.8 (4.1)	40.0 (8.2)
	SD	10.8	11.6	16.5
	MEDIAN	26.0	31.0	43.0
	RANGE	21 to 50	17 to 52	20 to 54
GENDER	N	6	8	4
	MALE	4 (66.7%)	6 (75.0%)	1 (25.0%)
	FEMALE	2 (33.3%)	2 (25.0%)	3 (75.0%)
RACE	N	6	8	4
	CAUCASIAN	4 (66.7%)	6 (75.0%)	4 (100.0%)
	AFRICAN AMERICAN	1 (16.7%)	2 (25.0%)	0 (0.0%)
	NATIVE AMERICAN	0 (0.0%)	0 (0.0%)	0 (0.0%)
	ASIAN/PACIFIC ISLANDER	0 (0.0%)	0 (0.0%)	0 (0.0%)
	OTHER	1 (16.7%)	0 (0.0%)	0 (0.0%)
ETHNICITY	N	6	8	4
	HISPANIC OR LATINO	2 (33.3%)	0 (0.0%)	1 (25.0%)
	NOT HISPANIC OR LATINO	4 (66.7%)	8 (100.0%)	3 (75.0%)

Program: 14.1.2.demog.sas
TABLE 14.1.2 – DEMOGRAPHICS

Renal parameters

Data are presented in

Table 5,

Table 6, Table 7, Figure 10 and Figure 11.

Table 5 Plasma Gb3 Concentration (LOCF) – Overall Population by Dose

PLASMA LYSO-GB3 (ng/ml)		PRX-102 0.2 mg/kg N = 6	PRX-102 1.0 mg/kg N = 6	PRX-102 2.0 mg/kg N = 4	Overall N = 16
DAY 0 (BASELINE)	N	6	6	4	16
	MEAN (SE)	93.9 (39.3)	70.6 (31.3)	20.2 (13.9)	66.7 (19.5)
	SD	96.2	76.7	27.9	78.0
	MEDIAN	75.6	47.6	7.9	40.5
	RANGE	(8, 273)	(5, 193)	(3, 62)	(3, 273)
WEEK 26	N	6	6	4	16
	MEAN (SE)	46.3 (20.5)	28.6 (10.0)	10.2 (5.7)	30.6 (8.9)
	SD	50.3	24.4	11.4	35.8
	MEDIAN	30.4	29.3	5.1	20.4
	RANGE	(7, 142)	(3, 70)	(3, 27)	(3, 142)
CHANGE FROM BASELINE TO WEEK 26	N	6	6	4	16
	MEAN (SE)	-47.6 (20.3)	-42.0 (23.7)	-10.1 (8.2)	-36.1 (11.9)
	SD	49.7	58.1	16.5	47.5
	MEDIAN	-50.0	-24.2	-2.9	-19.4
	RANGE	(-131, 9)	(-124, 20)	(-35, 0)	(-131, 20)
PERCENT CHANGE FROM BASELINE TO WEEK 26	N	6	6	4	16
	MEAN (SE)	-23.5 (29.4)	-21.1 (32.7)	-30.8 (12.4)	-24.4 (15.8)
	SD	72.1	80.0	24.8	63.3
	MEDIAN	-47.7	-50.1	-35.1	-45.3
	RANGE	(-90, 116)	(-80, 137)	(-56, 3)	(-90, 137)
WEEK 52	N	6	6	4	16
	MEAN (SE)	34.2 (9.1)	18.9 (7.5)	10.7 (6.8)	22.6 (5.1)
	SD	22.4	18.3	13.6	20.3
	MEDIAN	32.6	12.2	5.0	17.5
	RANGE	(7, 70)	(3, 47)	(2, 31)	(2, 70)
CHANGE FROM BASELINE TO WEEK 52	N	6	6	4	16
	MEAN (SE)	-59.7 (30.8)	-51.6 (24.0)	-9.5 (7.2)	-44.1 (14.9)
	SD	75.5	58.8	14.4	59.4
	MEDIAN	-40.2	-35.5	-3.1	-19.2
	RANGE	(-203, -0)	(-147, -2)	(-31, -1)	(-203, -0)
PERCENT CHANGE FROM BASELINE TO WEEK 52	N	6	6	4	16
	MEAN (SE)	-43.4 (12.2)	-59.9 (7.1)	-40.4 (7.5)	-48.9 (5.7)
	SD	30.0	17.4	15.0	22.9
	MEDIAN	-54.1	-60.9	-41.3	-50.4
	RANGE	(-75, -5)	(-79, -38)	(-56, -24)	(-79, -5)

As expected in patients with Fabry Disease, a higher mean (±SE) concentration of plasma Gb3 was observed at baseline in male patients than female patients.

Table 6 Plasma Lyso Gb3 Concentration (LOCF) – Overall Population by Dose

PLASMA LYSO-GB3 (ng/ml)		PRX-102 0.2 mg/kg N = 6	PRX-102 1.0 mg/kg N = 6	PRX-102 2.0 mg/kg N = 4	Overall N = 16
DAY 0 (BASELINE)	N	6	6	4	16
	MEAN (SE)	93.9 (39.3)	70.6 (31.3)	20.2 (13.9)	66.7 (19.5)
	SD	96.2	76.7	27.9	78.0
	MEDIAN	75.6	47.6	7.9	40.5
	RANGE	(8, 273)	(5, 193)	(3, 62)	(3, 273)
WEEK 26	N	6	6	4	16
	MEAN (SE)	46.3 (20.5)	28.6 (10.0)	10.2 (5.7)	30.6 (8.9)
	SD	50.3	24.4	11.4	35.8
	MEDIAN	30.4	29.3	5.1	20.4
	RANGE	(7, 142)	(3, 70)	(3, 27)	(3, 142)
CHANGE FROM BASELINE TO WEEK 26	N	6	6	4	16
	MEAN (SE)	-47.6 (20.3)	-42.0 (23.7)	-10.1 (8.2)	-36.1 (11.9)
	SD	49.7	58.1	16.5	47.5
	MEDIAN	-50.0	-24.2	-2.9	-19.4
	RANGE	(-131, 9)	(-124, 20)	(-35, 0)	(-131, 20)
PERCENT CHANGE FROM BASELINE TO WEEK 26	N	6	6	4	16
	MEAN (SE)	-23.5 (29.4)	-21.1 (32.7)	-30.8 (12.4)	-24.4 (15.8)
	SD	72.1	80.0	24.8	63.3
	MEDIAN	-47.7	-50.1	-35.1	-45.3
	RANGE	(-90, 116)	(-80, 137)	(-56, 3)	(-90, 137)
WEEK 52	N	6	6	4	16
	MEAN (SE)	34.2 (9.1)	18.9 (7.5)	10.7 (6.8)	22.6 (5.1)
	SD	22.4	18.3	13.6	20.3
	MEDIAN	32.6	12.2	5.0	17.5
	RANGE	(7, 70)	(3, 47)	(2, 31)	(2, 70)
CHANGE FROM BASELINE TO WEEK 52	N	6	6	4	16
	MEAN (SE)	-59.7 (30.8)	-51.6 (24.0)	-9.5 (7.2)	-44.1 (14.9)
	SD	75.5	58.8	14.4	59.4
	MEDIAN	-40.2	-35.5	-3.1	-19.2
	RANGE	(-203, -0)	(-147, -2)	(-31, -1)	(-203, -0)
PERCENT CHANGE FROM BASELINE TO WEEK 52	N	6	6	4	16
	MEAN (SE)	-43.4 (12.2)	-59.9 (7.1)	-40.4 (7.5)	-48.9 (5.7)
	SD	30.0	17.4	15.0	22.9
	MEDIAN	-54.1	-60.9	-41.3	-50.4
	RANGE	(-75, -5)	(-79, -38)	(-56, -24)	(-79, -5)

Figure 10 Mean BLISS Score Reduction in Gb3 Inclusions in Renal Biopsies PTC following 6 Months Treatment

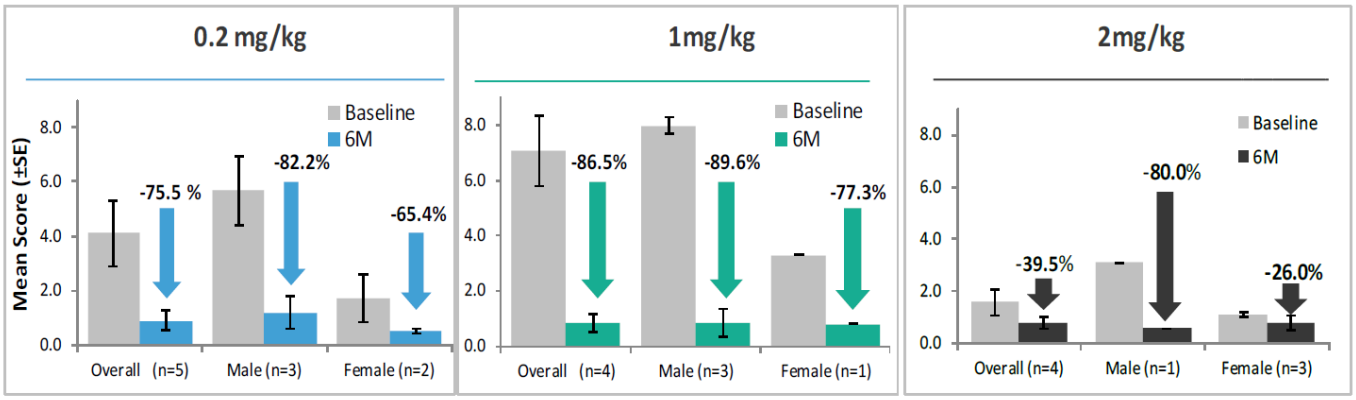


Figure 11 Mean eGFR (CKD-EPI) Levels (All versus Classic Fabry Patients)

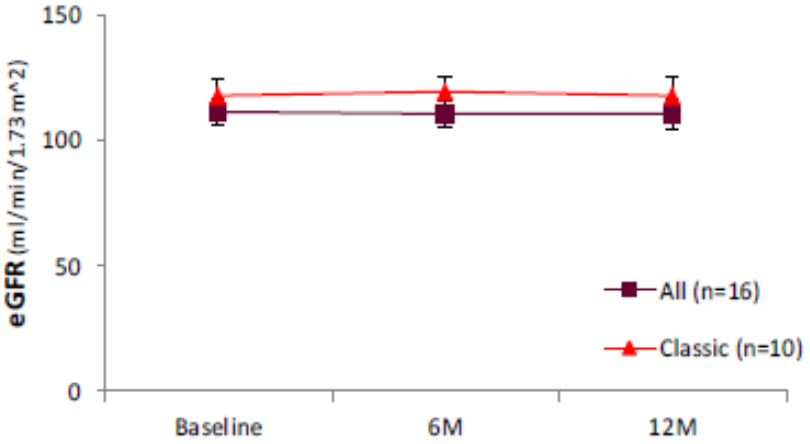


Table 7 GB3 Reduction in Peri-Tubular Kidney Capillaries (PTC) at Month 6 in PB-102-F01/F02 – Efficacy Population

Population	n	Gb3 Score per PTC (by BLISS) (±SE)				Responder Analysis % (n/n)	
		BL	6M	Change from BL to 6M	% Change from BL to 6M	≥ 20% Reduction in BLISS Score from BL to 6M	≥ 50% Reduction in BLISS Score from BL to 6M
All patients	14	4.0 (±0.8)	0.8 (±0.2)	-3.1 (±0.8)	-54.6 (±15.6)	85.7% (12/14)	78.6% (11/14)
All patients, excluding the patient with cardiac GLA variant	13	4.3 (±0.9)	0.8 (±0.2)	-3.4 (±0.8)	-67.8 (±8.9)	92.3% (12/13)	84.6% (11/13)
Classic	8	6.0 (±0.9)	0.9 (±0.3)	-5.1 (±0.8)	-84.1 (±3.4)	100% (8/8)	100% (8/8)

Cardiac parameters

At Month 12, male patients had a minor mean decrease from baseline in Left Ventricular Mass *(*LVM) in both 0.2 mg/kg and 1.0 mg/kg treatment groups. One patient who received 2.0 mg/kg had a slight increase in LVM from baseline. Male patients had also a minor mean decrease from baseline in Left Ventricular Mass Index (LVMI) in both the 0.2 mg/kg and 1.0 mg/kg treatment groups. One patient who received 2.0 mg/kg had also a slight increase in LVMI from baseline. Female patients had a marginal mean increase from baseline in LVMI in all three treatment groups. In addition, male patients had a minor mean decrease from baseline in Ejection Fraction (EF) in all three groups: 0.2 mg/kg (-2.6%, -4.7% mean percent decrease, n=4), 1.0 mg/kg (-10.1%, -15.2% mean percent decrease, n=4), and 2.0 mg/kg (-0.4%, -0.7% percent decrease, n=1). Female patients had a slight mean increase from baseline in EF in both the 0.2 mg/kg and 1.0 mg/kg groups; in contrast, the 2.0 mg/kg treatment group had a mean decrease from baseline.

Most of the assessed parameters using echocardiography (at screening and at Months 6 and 12) exhibited stable cardiac function after 12 months of treatment.

Brain MRI

The qualitative assessments of stroke at baseline and Month 12 showed there was no evidence of stroke detected in patient MRIs of the brain.

Mainz Severity Score Index (MSSI)

An overall slight reduction from baseline was demonstrated at Month 12 in the total general score (0.2 mg/kg, -2.8; 1.0 mg/kg, -1.2; 2.0 mg/kg, -1.5) of MSSI.

Gastrointestinal Symptoms Assessment (GSA) Questionnaire

Most patient responses to the GSA questionnaire showed a stable or favourable trend in the severity of abdominal pain, frequency of abdominal pain, and frequency of diarrhoea after 12 months of treatment.

Short Form Brief Pain Inventory (BPI)

At Month 12, patient responses to the BPI questionnaire showed a slight decrease from baseline severity score in pain and the pain interference score.

Study PB-102-F03

Up to 16 adult Fabry patients (males and females) (≥18 years) who completed study PB-102-F02 were planned to be included. A total of 15 adult patients (8 males and 7 females) were enrolled. Ten patients completed the study. Five patients discontinued from the study: three due to consent withdrawal, one due to a fatal, unrelated AE and one due to other reasons (pregnancy).

Renal parameters

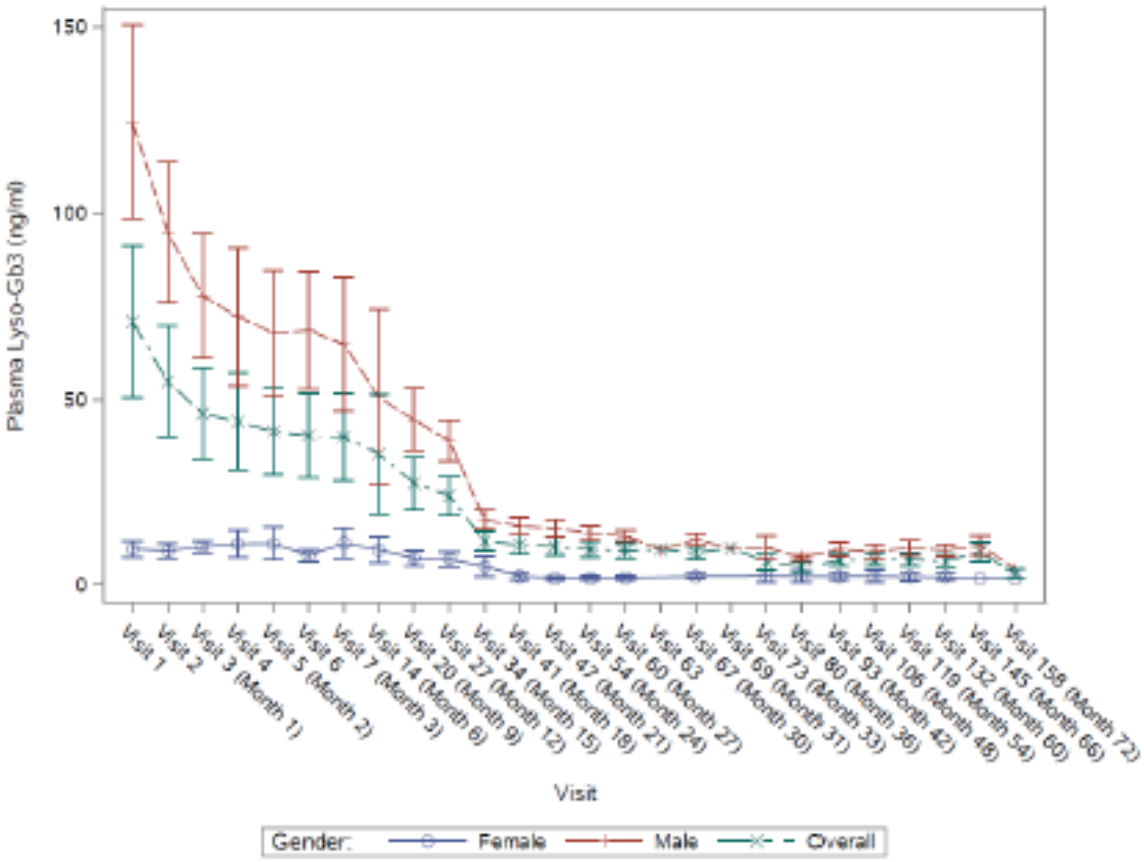
Data are presented in

Figure 12,

Table 8 and

Table 9.

Figure 12 Mean (SE) Plasma Lyso-Gb3 Concentrations Over Time, by Gender and Overall – Efficacy Population



Baseline values were those from baseline (i.e. Visit 1) in study PB-102-F01.
Source: Figure 15.2.2.

Table 8 Plasma Gb3 Concentration – Efficacy Population

Timepoints[1] Parameters		Male/Classic Patients N=8	Female/Non-classic Patients N=7	Treated ≥5 years N=10	Overall N=15
Baseline					
Absolute value (µg/mL)	n Mean (SE)	7 14.86 (1.9)	7 5.99 (0.4)	9 10.3 (1.5)	14 10.42 (1.5)
Month 12 (Visit 27)					
Absolute value (µg/mL)	n Mean (SE)	8 8.81 (0.8)	7 5.66 (0.6)	10 8.0 (0.8)	15 7.34 (0.6)
Change from baseline (µg/mL)	n Mean (SE)	7 -6.50 (1.8)	7 -0.33 (0.3)	9 -2.7 (1.1)	14 -3.41 (1.2)
% change from baseline	n Mean (SE)	7 -39.93 (8.1)	7 -5.82 (4.8)	9 -20.3 (7.3)	14 -22.88 (6.5)
Month 24 (Visit 54)					
Absolute value (µg/mL)	n Mean (SE)	7 9.31 (0.7)	4 6.35 (0.7)	10 8.1 (0.7)	11 8.24 (0.7)
Change from baseline (µg/mL)	n Mean (SE)	6 -6.18 (2.3)	4 0.13 (1.0)	9 -2.4 (1.3)	10 -3.66 (1.7)
% change from baseline	n Mean (SE)	6 -34.21 (12.4)	4 4.64 (14.6)	9 -14.0 (11.1)	10 -18.67 (11.0)
Month 48 (Visit 106)					
Absolute value (µg/mL)	n Mean (SE)	6 6.33 (0.5)	3 6.23 (1.5)	9 6.3 (0.5)	9 6.30 (0.5)
Change from baseline (µg/mL)	n Mean (SE)	5 -7.26 (1.6)	3 0.47 (1.4)	8 -4.4 (1.8)	8 -4.36 (1.8)
% change from baseline	n Mean (SE)	5 -51.17 (8.3)	3 7.02 (23.2)	8 -29.3 (14.0)	8 -29.35 (14.0)
Month 60 (Visit 132)					
Absolute value (µg/mL)	n Mean (SE)	6 6.58 (0.6)	4 8.40 (1.2)	10 7.3 (0.6)	10 7.31 (0.6)
Change from baseline (µg/mL)	n Mean (SE)	5 -6.72 (1.4)	4 2.18 (1.6)	9 -2.8 (1.9)	9 -2.77 (1.9)
% change from baseline	n Mean (SE)	5 -47.90 (7.4)	4 40.66 (26.9)	9 -8.5 (19.4)	9 -8.54 (19.4)

Baseline values were those from baseline (i.e. Visit 1) in study PB-102-F01 and the presented timepoints correspond to an overall maximum treatment period of 72 months with PRX-102 (i.e. 3 months in study PB-102-F01, 9 months in study PB-102-F02 and 60 months in study PB-102-F03).
Source: Table 14.2.2.1, Table 14.2.2.2, and Table 14.2.2.3.

Table 9 eGFR (ml/min/1.73 m²) – Efficacy Population

Timepoints[1] Parameters		Male/Classic Patients N=8	Female/Non-classic Patients N=7	Treated ≥5 years N=10	Overall N=15
Baseline					
Absolute value	n	8	7	10	15
	Mean (SE)	118.1 (7.7)	104.4 (7.5)	107.9 (6.0)	111.7 (5.5)
Month 12 (Visit 27)					
Absolute value	n	8	6	10	14
	Mean (SE)	117.1 (9.0)	101.1 (9.6)	108.5 (7.7)	110.3 (6.7)
Change from baseline	n	8	6	10	14
	Mean (SE)	-1.0 (3.0)	-1.1 (3.2)	0.6 (2.4)	-1.0 (2.1)
Month 24 (Visit 54)					
Absolute value	n	7	4	10	11
	Mean (SE)	110.2 (6.4)	101.1 (10.9)	107.5 (6.1)	106.9 (5.5)
Change from baseline	n	7	4	10	11
	Mean (SE)	-2.5 (0.9)	3.1 (2.4)	-0.3 (1.4)	-0.4 (1.3)
Month 48 (Visit 106)					
Absolute value	n	6	4	10	10
	Mean (SE)	105.9 (4.2)	97.1 (12.0)	102.4 (5.2)	102.4 (5.2)
Change from baseline	n	6	4	10	10
	Mean (SE)	-8.6 (4.6)	-0.9 (5.7)	-5.5 (3.6)	-5.5 (3.6)
Month 60 (Visit 132)					
Absolute value	n	6	4	10	10
	Mean (SE)	100.0 (8.3)	92.4 (11.4)	97.0 (6.4)	97.0 (6.4)
Change from baseline	n	6	4	10	10
	Mean (SE)	-14.5 (1.7)	-5.6 (2.6)	-10.9 (2.0)	-10.9 (2.0)

Baseline values were those from either baseline (i.e. Visit 1) or screening (whichever was the latest observation) in study PB-102-F01 and the presented timepoints correspond to an overall treatment period of 72 months with PRX-102 (i.e. 3 months in study PB-102-F01, 9 months in study PB-102-F02 and 60 months in study PB-102-F03).
Source: Table 14.2.4.1.1, Table 14.2.4.1.2, and Table 14.2.4.1.3

The mean (SE) annualized eGFR slope was -1.6 mL/min/1.73 m²/year (0.8). The mean annualized eGFR slopes were more negative (indicating a higher decrease in eGFR over time) in male versus female patients and in long-term treated patients compared to the overall population.

Cardiac parameters

The mean (SE) LVM absolute value was considered to be within normal ranges. the mean (SE) absolute LVM values remained normal at Month 24 and Month 60. The mean (SE) LVMI absolute value was considered to be within normal ranges for male patients and below normal for female patients. A slow increase in mean LVMI was observed in the course of the study in both subgroups, with larger increases in female patients. At Month 60, the male patients remained within normal levels throughout, and the increase in LVMI in female patients led to a mean value at Month 60 also within normal ranges. The mean (SE) EF absolute value was considered to be within normal ranges and remained stable over 60 months of treatment.

No relevant changes from screening were observed during the study in the echocardiography parameters. Most patients had normal parameters throughout the study.

Mainz Severity Score Index

Mean (SE) change from baseline in the overall score was -7.5 (1.8) at month 24 and -3.6 (2.3) at month 60. All mean sub-scores showed decreases.

Fabry Clinical Events

A single Fabry Clinical Event was identified; a non-cardiac-related death following a COPD exacerbation in a patient after 39 months of treatment. The event was considered unrelated to study treatment.

Gastrointestinal Symptoms Assessment Questionnaire

Post-baseline evaluations generally showed a reduction of the number of patients with moderate or severe abdominal pain except at month 48 where high levels of moderate pain were reported. Reporting rates for frequencies of abdominal pain and diarrhoea fluctuated in the course of the study. No clear trends were observed for the proportions of patients with symptoms over time.

2.6.5.2. Main study(ies)

STUDY PB-102-F20

PB-102-F20, the main Phase 3 study, is a randomised, double-blind, active control designed to investigate the safety and efficacy of pegunigalsidase alfa compared to Fabrazyme (algasidase beta) and assess renal function in adult patients with Fabry disease previously treated with Fabrazyme (algasidase beta).

Methods

- Study Participants

Main inclusion criteria were:

1. Symptomatic adult Fabry disease patients, age 18–60 years

2. Males: Plasma and/or leucocyte alpha galactosidase activity (by activity assay) less than 30% mean normal levels and one or more of the characteristic features of Fabry disease: neuropathic pain, cornea verticillata, clustered angiokeratoma

3. Females:

a. historical genetic test results consistent with Fabry pathogenic mutation and one or more of the described characteristic features of Fabry disease: neuropathic pain, cornea verticillata, clustered angiokeratoma

b. or in the case of novel mutations a first-degree male family member with Fabry disease with the same mutation, and one or more of the characteristic features of Fabry disease: neuropathic pain, cornea verticillata, clustered angiokeratoma

4. Screening eGFR by CKD-EPI equation 40 to 120 mL/min/1.73 m^2

5. Linear slope of eGFR more negative than -2 mL/min/1.73 m^2, based on at least 3 serum creatinine values over approximately 1 year (range of 9 to 18 months, including the value obtained at the screening visit)

6. Treatment with a dose of 1 mg/kg agalsidase beta per infusion every 2 weeks for at least one year. Over the last 6 months, the dose had to have been stable and the patient had to have received at least 80% of the total amount (i.e., at least 10.4 of 13 infusions).

Main exclusion criteria were:

1. History of anaphylaxis or Type 1 hypersensitivity reaction to agalsidase beta

2. Known non-pathogenic Fabry mutations (polymorphism)

3. History of renal dialysis or transplantation

4. History of acute kidney injury in the 12 months prior to screening,

5. Angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) therapy initiated or dose changed in the 4 weeks prior to screening

6. Patient with a screening eGFR value of 91-120 mL/min/1.73 m^2: having an historical eGFR value higher than 120 mL/min/1.73 m^2 (during 9 to 18 months before screening)

7. Urine protein to creatinine ratio (UPCR) > 0.5 gr/gr (0.5 mg/mg or 500 mg/g) and not treated with an ACE inhibitor or ARB

8. Cardiovascular event (myocardial infarction, unstable angina) in the 6-month period before randomization

9. Congestive heart failure NYHA Class IV

10. Cerebrovascular event (stroke, transient ischemic attack) in the 6-month period before randomization

- Treatments

Patients were randomized in a 2:1 ratio to receive either pegunigalsidase alfa or agalsidase beta. Both products were administered at the same dosage and on the same schedule: 1 mg of drug per kg of body weight, once every two weeks by intravenous infusion. There was no treatment adjustment in the group of patients previously treated with agalsidase beta.

- Objectives

At initial submission, an interim report collecting up to the date of the last patient's 12-month visit was presented. During the procedure, the final report with data up to 24 months for all participants was submitted.

The aim of the interim analysis was to demonstrate that in terms of eGFR slope, pegunigalsidase alfa is non-inferior to agalsidase beta by a non-inferiority margin of 3.0 ml/min/1.73 m^2/year. Secondary objectives were to determine effect of pegunigalsidase alfa over agalsidase beta on use of pain medication, estimated glomerular filtration rate (eGFR); UPCR; plasma Gb3, plasma lyso-Gb3, urine lyso-Gb3; Short Form Brief Pain Inventory; Quality of Life; Mainz Severity Score Index; cardiac stress test; echocardiogram, and cardiac MRI.

The aim of the final 2 years analysis was initially planned to demonstrate superiority of pegunigalsidase alfa over agalsidase beta to support the submission of another regulatory authority. In January 2022, the applicant modified the objective for the final analysis from superiority to non-inferiority testing.

- Outcomes/endpoints

The primary endpoint was change from baseline in measure of eGFR slope.

Secondary endpoints are the change from baseline in other efficacy measures; determination of PK parameters (subset of patients only), biomarkers of Fabry disease, and evaluation of safety measures.

Table 10 Efficacy measures:

Changes from baseline in measures of kidney function	eGFR as determined by serum creatinine	Screening, baseline and then monthly
	eGFR slope (primary endpoint)	
	Urine protein to creatinine ratio (UPCR)	Screening, baseline and then every 3 months
Changes from baseline in measures of cardiac morphology and function	Cardiac MRI	Baseline and then every 12 months
	Cardiac stress test	
	Electrocardiogram	
Changes from baseline in biomarkers of Fabry disease	Plasma lyso-Gb3 concentration	Baseline, 1.5 months, every 3 months up to a year and then every 6 months up to 2 years
	Urine lyso-Gb3 concentration	
	Plasma Gb3 concentration	
Changes from baseline in severity of Fabry disease	Mainz Severity Score Index (MSSI)	Baseline and then every 6 months
Changes from baseline in measures of pain	Short Form Brief Pain Inventory (BPI)	Screening, baseline and then every 3 months
	Use of pain medication	Every visit
Changes from baseline in measure of overall well-being	Quality of life questionnaire (EQ-5D-5L)	Baseline and then every 6 months
Incidence of Fabry Clinical Events	Includes serious renal events, cardiac events, cerebrovascular events, and non-cardiac-related death	Throughout study
Achievement of Fabry Kidney Disease therapeutic goals	As per the European Expert Consensus Statement on Therapeutic Goals in Fabry Disease	Throughout study

- Sample size

Interim Analysis – Non inferiority testing

With a total of approximately 66 patients in a 2:1 randomization ratio, there was at least 90% power to demonstrate the non-inferiority of pegunigalsidase alfa vs. agalsidase beta in terms of mean annualized change (slope) in eGFR. The power was computed assuming a one-sided two-sample t-test with a one-sided alpha level of 0.025 and a non-inferiority margin of -3.0 mL/min/1.73 m2/year. The true difference in slopes was assumed to be 1.1 mL/min/1.73 m2/year in favour of pegunigalsidase alfa, with the standard deviation of the slopes being 1.5 mL/min/1.73 m²/year in each group.

Final Analysis – Superiority testing (as initially planned)

With a total of approximately 66 patients in a 2:1 randomization ratio, there will be approximately 80% power to detect a difference of 1.1 mL/min/1.73 m²/year between the mean annualized changes (slope) in the two arms. The power was computed assuming a two-sample t-test at a two sided alpha level of 0.05 and a standard deviation of the slopes of 1.5 mL/min/1.73 m²/year in each group. For this analysis, the null hypothesis is that the difference in mean annualized change in eGFR between the treatment groups is 0,

versus an alternative hypothesis that it is not 0. Based on previous research, the mean annualized change (slope) in eGFR in patients treated with agalsidase beta is -3.0 mL/min/1.73 m²/year. A 1.1 reduction in the rate of decline in renal function (improvement) is anticipated to be equal to a mean annualized change (slope) in eGFR with pegunigalsidase alfa of -1.9 mL/min/1.73 m²/year representing an approximately 30% improvement, which would be considered clinically relevant.

To allow for a dropout rate as high as 15%, it was planned for approximately 78 patients to be randomized: approximately 52 patients to the PRX-102 arm and approximately 26 to the agalsidase beta arm. This was expected to yield approximately 44 and 22 completers in the two arms, respectively.

These power calculations were based on a two-sample t-test, and were performed using the PASS-13 Tests for Two Means procedure.

- Randomisation and Blinding (masking)

A fixed block randomization list, stratified at baseline by urine protein to creatinine ratio (UPCR, was generated with a 2:1 randomization (PRX-102:agalsidase beta) and incorporated into the system. Once patient eligibility was confirmed by the sponsor's Medical Monitor, the system generated a subject randomization ID number. The investigative staff and patients were blinded to the treatment assignment.

Since PRX-102 and agalsidase beta differ in appearance and packaging, the individual doses for infusion were prepared by an unblinded pharmacist or nurse at the site, resulting in identical infusion bag appearance and blinded labelling prior to administration.

Both the patients and the staff members administering the treatments were blinded as to what the infusion bag contained.

- Statistical methods

Analysis populations

The following populations were used for tables summarizing efficacy and safety:

• The Intent to Treat (ITT) population consisted of all randomized patients who received at least one dose (including a partial dose) of study medication (either pegunigalsidase alfa or agalsidase beta), and was based on the assigned treatment arm in the randomization.

• The Per Protocol (PP) population included all ITT patients who completed at least 12 months of treatment, with study drug compliance of at least 80%, and with no major protocol violations that could have impacted the primary endpoint. The PP is considered as a supportive analysis for the primary efficacy analysis; however, for the non-inferiority analysis of this interim report, the ITT and PP populations have a similar weight in the interpretation of the results.

• The Safety population consisted of all patients who were randomized and who received at least one partial dose of study medication. Assignment was by actual treatment received.

Primary endpoint analyses

Four different statistical approaches were considered, in order to compare the annualized slope between the treatment arms. Within the framework of a longitudinal mixed model, two models were considered, a random intercept model (RI) and a random intercept random slope (RIRS) model.

Within the framework of a two-stage approach, the first stage includes estimation of individual slopes using linear regression. Two models were considered for the 2nd stage: Analysis of Covariance (ANCOVA) and quantile regression for comparing the median slopes.

For the interim analysis the models considered were the RIRS, RI and two-stage with ANCOVA.

The two-stage with quantile regression was added following a recent publication by Ortiz et al (2021) in which the median eGFR slope of Fabrazyme was compared to the median eGFR slope of un-treated Fabry patients. Unlike linear regression which makes distributional assumptions (i.e., normality), the quantile regression makes no such assumptions, and is robust against outliers

Following interaction with another regulatory authority, the two-stage with quantile regression on the ITT set was considered as the primary model for the final analysis. For non-inferiority, both the ITT and PP should be considered when interpreting the study, so the analysis will be performed also on the PP set.The two-stage with ANCOVA, RI, RIRS on the ITT and PP were considered as supportive. For all of the analyses time was measured relative to day of 1st infusion.

The primary analysis at the interim time point (when the last patient completes 1 year of treatment) is a non-inferiority analysis. The null hypothesis is that the difference in slopes between the two treatment arms is ≤ -3.0 mL/min/1.73 m²/year versus the alternative that it is larger. Non-inferiority will be declared if the lower bound of the 95% CI for the interaction term of treatment group by time is greater than -3.0 mL/min/1.73 m²/year, the pre-specified non-inferiority margin. If this criterion is met, then it implies that the slope of the eGFR in the PRX-102 treatment arm is not worse than -3.0 mL/min/1.73 m²/year compared to the slope in the agalsidase beta group. All available data will be used for the interim and final analyses, in particular, for the evaluation of non-inferiority which occurs after the last patient completes 1 year of treatment, all available data up to the cut-off date, from each patient will be used (i.e., data that extends beyond one year).

The primary analysis at the final time point (after the last enrolled patient has 2-years of treatment) was initially a superiority analysis. The null hypothesis is that the difference in slopes between the two treatment arms is 0 versus the two-sided alternative. Superiority will be declared if the coefficient associated with the time by treatment interaction is positive and significantly different than 0 at a two-sided level of 0.05, or equivalently if the lower bound of the confidence interval for the interaction term is greater than 0. In the submission (during the procedure) of the final study report, the objective of the final analysis was changed into a non-inferiority testing. As no previously established clinically relevant NI margin is available, the NI margin in this study was identified using the combined knowledge on the natural history of the disease and the data published on the effect of available treatments on renal function deterioration in Fabry Disease patients. As claimed by the applicant, the collective reported evidence supports the clinical choice of the -3 ml/min/1.73m2/year as the lower boundary of the CI also for this 2 years NI analysis for the following reasons:

- Evidence on the natural history of the disease suggests that untreated patients tend to present progressive kidney deterioration by showing an eGFR slope worse than -3 ml/min/1.73m2/year (from around -4 to -12 ml/min/1.73m2/year), therefore achieving -3 ml/min/1.73m2/year can be considered as a relevant threshold for assessing the benefit of a disease specific treatment.

- The European Therapeutic Goals published by Wanner (2018) has used the same threshold for defining patients considered clinically stable with regards to renal function (one of the main goals for long-life treatment of progressing diseases), thus confirming that -3 ml/min/1.73m2/year is a reasonable threshold from a clinical perspective.

- Finally, this threshold was pre-defined in Version 1.0 of the study protocol and used for the first 12 months NI interim analysis, mainly on the basis of what was already known on the natural history of the disease and on what is published in literature on the treatments effect. Considering that the slope is an annualized measure the use of the same margin is considered an appropriate approach (since the NI hypothesis is tested on the same population, with additional data).

Sensitivity/supportive analyses for robustness on primary endpoint

The sensitivity analyses used the same estimand as the primary analysis but differed in the covariates used. The supportive analyses used different estimands (mean using RIRS, RI, and two-stage with ANCOVA; mean eGFR change from baseline using a Mixed Model Repeated Measure (MMRM) and other quantiles using quantile regression).

Sensitivity analyses included: a two-stage with quantile regression was repeated including the stratification factor (UPCR < 1; ≥ 1 gr/gr), analysis with exclusions of any eGFR value associated with events of an elevated serum creatinine, defined by a 1.5-fold increase or greater compared to the immediate previous serum creatinine value as long as that measurement was taken no more than 34 days before.

For the interim report, further exploration of the group difference in eGFR slope was done through several pre-specified sensitivity analyses: a 2-stage sensitivity analysis, in which the first stage calculated the slopes for each patient and the second stage compared the treatment arms with ANCOVA using those slopes; a random intercept model, which is similar to the random intercept random slope model used for the main analysis; and a Mixed Model Repeated Measure (MMRM) model.

Supportive analyses included: A RIRS longitudinal mixed model used to compare the eGFR slopes of the two groups, a two-Stage Approach Using Analysis of Covariance (The 1st stage of this approach is identical to the 1st stage of the two-stage with quantile regression. At the next stage, the eGFR slope between the two treatment arms was compared using ANCOVA. The dependent variable was the slope of each individual patient and the model included the same covariates as the primary analysis); Random Intercept (RI) Longitudinal Model (similar to the RIRS Longitudinal Model, but the random effect included only the intercept as random component); MMRM analysis using the eGFR change from baseline (response variable was the change from Baseline in eGFR at each scheduled study visit where eGFR was measured. The model included the following covariates: intercept; Baseline eGFR; Visit; Treatment arm and visit by treatment interaction. Non-inferiority was declared if the lower bound of the 95% CI for the contrast between treatment arms at the 2-year visit (Week 104) is greater than or equal to -6.0 mL/min/1.73 m2/year) and a two-stage with quantile regression with additional quantiles (analysis repeated for the ITT and PP, where in the 2nd stage the 25th and 75th quantiles as well as their difference was estimated instead of the median).

The sensitivity and supportive analyses as described were performed for ITT and PP sets and summarized graphically with a Forest plot. Evaluation of non-inferiority was done similar to the primary analysis.

Sensitivity analyses for Missing Data

The primary analysis and the sensitivity analyses assume that the missing data were Missing At Random (MAR). Multiple Imputation (MI) was used to assess the impact of missing data. To this end MI under the

MAR assumption was conducted for patients who early terminated. Missing data were imputed within each treatment arm.

Multiplicity issues

The primary efficacy endpoint for both interim and final analyses is the mean annualized change (slope) in estimated glomerular filtration rate (eGFR). The interim analysis is a non-inferiority analysis and the final analysis was a superiority analysis (as initially planned). As claimed by the applicant, for regulatory purposes, demonstration of non-inferiority of PRX-102 compared to agalsidase beta at 12 months to support the EU submission and superiority at 24 months to support the submission of another regulatory authority will be considered trial success.

The interim analysis occurs after the last patient randomized has completed 12 months of treatment The non-inferiority assessment will be evaluated at a one-sided alpha level of 0.025. The final analysis will be conducted when all patients complete 24 months of treatment. This superiority assessment will be performed using a two-sided alpha level of 0.05. Since there is no intention to stop the study for futility or efficacy and no plan for study adaptations based on outcomes of the interim analysis, no alpha penalty for the final superiority analysis is needed.

Subgroup Analysis

Subgroup analyses will be conducted based on baseline characteristics and demographics for selected efficacy and safety endpoints and only if the size of each of the groups is at least 10 (combined over the two treatment arms). In case of a substantial overlap between different sub-groups (e.g. up to 2 patients who differ), one of these subgroups may be skipped.

The selection of subgroup will be from the following list:

- Gender (Male or Female)

- Anti-Drug Antibodies (ADA) status at baseline (Negative; Positive). Determination of status is based on Immunoglobulin G (IgG) positive at baseline (Section 11.7). For patients who were randomized to PRX-102 arm, their ADA status at for PRX-102 at baseline will be used. For patients who were randomized to agalsidase-beta arm, their ADA status at for agalsidase-beta at baseline will be used. In case the test for baseline visit is missing, then the result from the screening visit will be used.

- Fabry Disease (FD) classification (Classic/Non-Classic). In order to be classified as FD classic, a patient should have ≤ 5% mean of lab normal ranges residual enzymatic activity in plasma or leukocytes at baseline visit and at least one Fabry specific symptom: Cornea Verticillata, Acroparesthesias, Angiokeratomas.

- Baseline eGFR (≤ 60; 60 < and ≤ 90; > 90 mL/min/1.73m²)

- Annualized slope of eGFR (≤ -5; > -5 mL/min/1.73m²/year)

- Use of ACEi or ARB treatment at baseline (Yes/No). The usage of ACEi or ARB is based on classification in the medication form in the eCRF. Based on this form, it is possible to identify patients who received ACEi or ARB at Baseline date. All other patients will be classified as “No” for this subgroup.

- Region (United States (US)/ex-US)

- UPCR categories (≤ 0.5 gr/gr; 0.5 < and < 1 gr/gr; ≥1 gr/gr).

Changes in SAP (January 2022) for final analysis

As presented by the applicant, the following changes were made in SAP, mainly following regulatory authority interactions:

- The following endpoints were added: Infusion-related reactions (IRRs), occurrence of Fabry clinical events, and achievement of Fabry Kidney Disease Therapeutic Goals.

- Analysis sets: The protocol stated that a patient who received at least one complete dose of study product would be included in the ITT population; the SAP changed this to also include receipt of at least one partial dose. The Per Protocol population was defined in the protocol to include patients who completed the study with no major protocol deviations; the SAP added a compliance requirement and refined the type of major protocol deviations to be excluded.

- The primary analysis of the study was changed from superiority to non-inferiority, as demonstrating superiority was no longer required.

- The primary analysis was changed to the two-stage with quantile regression, and the RIRS (primary analysis for the interim analysis) is now a supportive analysis. In addition to estimating the median slope, quantile regression was used to estimate the 25th and 75th quantiles as supportive analysis.

- UPCR was removed from all models (RIRS, RI, 2-stage with ANCOVA, MMRM for eGFR change from BL and for lyso-Gb3 change from BL). Quantile regression for the median which includes UPCR as a covariate was presented as a sensitivity analysis.

- The approach for multiplicity control

- The only shift table for laboratory values was for changes in ADA status. No other shift tables are provided, as no other safety laboratory values are of special interest.

- Evaluation of Acute Kidney Injury is based on AE reporting and clinical judgment of the investigator, not as described in the protocol.

- The statistical section of the protocol had stated that hypersensitivity was to be analyzed as an AE of special interest. Since IRRs were added as an endpoint (see first item in this list), hypersensitivity were evaluated as part of IRRs, being a wider category.

Post-hoc analyses

To further investigate the impact of the imbalances at baseline for the baseline characteristics, the primary analysis was repeated with the following parameters added as covariate to the primary analysis model: Gender, Fabry disease classification and ADA status at baseline.

Results

- Participant flow

A total of 127 prospective subjects were assessed for inclusion. Of these, 49 failed screening and 78 were enrolled and randomized. The reasons for screen failure were not meeting all the inclusion/exclusion criteria (n=39), withdrawal of consent prior to randomization (n=3), and “other” (n=7).

In the interim analysis, of the 78 randomized patients, 53 were assigned to the pegunigalsidase arm and 25 to the agalsidase beta arm. All but 1 patient in the pegunigalsidase alfa arm received at least 1 dose of study product. During the first year of treatment, 3 patients in the pegunigalsidase alfa arm terminated the study; and up to the cut-off date for the interim analysis during the second year, 1 subject from each arm discontinued.

At the time of the cut-off date, 27 (50.9%) and 15 (60%) patients, respectively, had completed the full 24 months. Reasons for discontinuation were adverse event (2 patients in the pegunigalsidase alfa arm, none in the agalsidase beta arm) and voluntary withdrawal (3 and 1, respectively). One of the adverse events that led to withdrawal, a drug hypersensitivity reaction, was considered related to study product.

In the final analysis, of the 78 randomized patients, 53 were assigned to the pegunigalsidase alfa and 25 to the agalsidase beta arm. All but 1 patient in the pegunigalsidase alfa arm received at least 1 dose of study product. A total of 5 patients in the pegunigalsidase alfa arm and 1 patient in the agalsidase beta arm terminated the study prematurely while 48 (90.6%) and 24 (96.0%) patients, respectively, completed the 24-month study period. Reasons for discontinuation were AE (2 patients in the pegunigalsidase alfa arm, none in the agalsidase beta arm) and voluntary withdrawal (3 and 1, respectively). One of the AEs that led to withdrawal, a drug hypersensitivity reaction, was considered related to study treatment.

Restrictions related to COVID-19 impacted some patients who were still enrolled in the study during the pandemic and could not attend site visits. Some visits had to be rescheduled, which in a few cases caused the duration of participation to be prolonged beyond Week 104. During this time, these patients continued to receive treatment. Prolongation for this reason occurred for 4 patients: 3 in the pegunigalsidase alfa arm and 1 in the agalsidase beta arm.

Table 11 Patient disposition – Enrolled set

	PRX-102 n (%)	Agalsidase Beta n (%)	Overall n (%)
Randomized	53	25	78
Exposed	52 (98.1)	25 (100.0)	77 (98.7)
Completed 12 months	49 (92.5)	25 (100.0)	74 (94.9)
Completed 24 months	27 (50.9)	15 (60.0)	42 (53.8)
Discontinued [1]	5 (9.4)	1 (4.0)	6 (7.7)
Reason for discontinuation:			
Adverse event	2 (3.8)	0 (0.0)	2 (2.6)
Withdrawal of consent	3 (5.7)	1 (4.0)	4 (5.1)
Study prolongation due to COVID-19 restrictions	3 (5.7)	1 (4.0)	4 (5.1)

A total of 704 protocol deviations occurred in the study. All deviations, both major and minor (based on the deviation plan definitions). Of the 77 analyzed patients, 53 (68.8%) had at least one major deviation, with the rates almost the same in the two treatment groups. The most common type of deviation was in the category of study procedures criteria, reported in 23 (44.2%) pegunigalsidase alfa patients and 8 (32.0%) agalsidase beta patients, followed by laboratory assessment criteria in 18 (34.6%) and 9 (36.0%), respectively, and visit schedule criteria in 16 (30.8%) and 6 (24.0%), respectively.

Of the 77 treated patients, 55 (71.4%) had at least one critical or major deviation, with similar rates in the two treatment groups. The most common type of deviation was in the category of study procedures criteria, reported in 24 (46.2%) pegunigalsidase alfa patients and 8 (32.0%) agalsidase beta patients, followed by laboratory assessment criteria in 22 (42.3%) and 9 (36.0%), respectively, and visit schedule criteria in 16 (30.8%) and 6 (24.0%), respectively.

Table 12 Summary of critical or major protocol violations – ITT set- *interim analysis*

	PRX-102 N=52 n (%)	Agalsidase Beta N=25 n (%)	Overall N=77 n (%)
Number of subjects with at least one critical or major violation	36 (69.2)	17 (68.0)	53 (68.8)
Study procedures criteria	23 (44.2)	8 (32.0)	31 (40.3)
Laboratory assessment criteria	18 (34.6)	9 (36.0)	27 (35.1)
Visit schedule criteria	16 (30.8)	6 (24.0)	22 (28.6)
IP compliance	5 (9.6)	4 (16.0)	9 (11.7)
Eligibility and entry criteria	5 (9.6)	2 (8.0)	7 (9.1)
Informed consent	3 (5.8)	1 (4.0)	4 (5.2)
Source document criteria	1 (1.9)	1 (4.0)	2 (2.6)
Other criteria	2 (3.8)	0 (0.0)	2 (2.6)
Administrative criteria	0 (0)	1 (4.0)	1 (1.3)
Serious adverse event criteria	1 (1.9)	0 (0.0)	1 (1.3)

Summary of critical or major protocol violations – ITT set final analysis

	PRX-102 N=52 n (%)	Agalsidase Beta N=25 n (%)	Overall N=77 n (%)
Number of subjects with at least one critical or major deviation	*38 (73.1)*	*17 (68.0)*	*55 (71.4)*
Study procedures criteria	24 (46.2)	8 (32.0)	32 (41.6)
Laboratory assessment criteria	22 (42.3)	9 (36.0)	31 (40.3)
Visit schedule criteria	16 (30.8)	6 (24.0)	22 (28.6)
IP compliance	5 (9.6)	4 (16.0)	9 (11.7)
Eligibility and entry criteria	5 (9.6)	2 (8.0)	7 (9.1)
Informed consent	5 (9.6)	2 (8.0)	7 (9.1)
Source document criteria	1 (1.9)	1 (4.0)	2 (2.6)
Administrative criteria	1 (1.9)	1 (4.0)	2 (2.6)
Other criteria	2 (3.8)	0 (0.0)	2 (2.6)
SAE criteria	1 (1.9)	0 (0.0)	1 (1.3)

Note: Subjects can have more than one critical or major protocol violation of the same type
Source: Table 14.1.3

Three patients had deviations that were considered critical:

• Laboratory assessment criteria: one patient in the pegunigalsidase alfa arm had blood drawn for PK assessment at a home visit despite having declined to take part in the optional PK assessments (the sample did not undergo testing).

• Serious adverse event criteria: one patient in the pegunigalsidase alfa arm experienced a serious adverse event (hospitalization for hypothermia) that was not reported within 24 hours of the site becoming aware of it, as planned in the protocol.

• Administrative criteria: one patient in the agalsidase beta arm had some vital signs taken by a study staff member who was unblinded as to which product had been administered.

- Recruitment

Date of first patient informed consent: 22 August 2016

Date of Month 12 visit for last patient: 12 October 2020

Date of last visit for last patient: 12 October 2021

- Conduct of the study

The original protocol, Version 1 dated 26 January 2016, was amended 4 times (Version 2 dated 05 April 2016, Version 3 dated 26 May 2016, Version 4 dated 29 September 2016 and Version 5 dated 14 July 2017). Versions 1 and 2 were submitted to the another regulatory authority, but were not implemented; the first patient was enrolled under Version 3.

- Baseline data

Demographic data for the ITT population are shown in

Table 13. Age was similar across the arms, with an overall mean of 44.3 years and a range of 18 to 60 years. Males outnumbered females in both arms, with the disparity greater in the agalsidase beta arm (72% vs. 28%). (As mandated in the protocol, enrolment of females could not exceed 50%). The majority of participants were white (93.5%) and non-Hispanic/Latino (97.4%).

Table 13 Summary of demographics data at baseline – ITT set

	PRX-102 N=52	Agalsidase Beta N=25	Overall N=77
Age (years)			
Mean (standard error)	43.9 (1.4)	45.2 (1.9)	44.3 (1.1)
Standard deviation	10.2	9.6	10.0
Median	44.0	48.0	46.0
Minimum, maximum	20, 60	18, 58	18, 60
Gender: n (%)			
Male	29 (55.8)	18 (72.0)	47 (61.0)
Female	23 (44.2)	7 (28.0)	30 (39.0)
Race: n (%)			
Asian	2 (3.8)	0	2 (2.6)
Black or African American	1 (1.9)	2 (8.0)	3 (3.9)
White	49 (94.2)	23 (92.0)	72 (93.5)
Ethnicity: n (%)			
Hispanic or Latino	0	2 (8.0)	2 (2.6)
Not Hispanic or Latino	52 (100.0)	23 (92.0)	75 (97.4)

Baseline measures associated with kidney function are presented in Table 14.

Table 14 Baseline efficacy measures associated with kidney function – ITT set

	PRX-102 N=52	Agalsidase Beta N=25	Overall N=77
eGFR (mL/min/1.73 m²)			
Mean (SE)	73.25 (2.75)	73.49 (4.04)	73.33 (2.26)
SD	19.84	20.20	19.82
Median	73.45	74.85	74.51
Min, Max	30.2; 125.9	34.1; 107.6	30.2; 125.9
eGFR Category (mL/min/1.73 m²), n (%)			
≤ 60	13 (25.0%)	8 (32.0%)	21 (27.3%)
60 < and ≤ 90	28 (53.8%)	12 (48.0%)	40 (51.9%)
>90	11 (21.2%)	5 (20.0%)	16 (20.8%)
eGFR slope at screening (mL/min/1.73 m²/year) [1]			
Mean (SE)	-8.42 (0.96)	-7.79 (0.95)	-8.22 (0.72)
SD	6.96	4.74	6.30
Median	-6.10	-5.97	-6.07
Min; Max	-32.7; -2.1	-19.5; -2.3	-32.7; -2.1
eGFR slope at baseline (mL/min/1.73 m²/year) [2]			
Mean (SE)	-8.07 (0.91)	-8.48 (0.83)	-8.21 (0.67)
SD	6.59	4.14	5.88
Median	-7.10	-8.21	-7.32
Min; Max	-30.5; 6.3	-20.3; -2.8	-30.5; 6.3
Baseline eGFR slope categories (mL/min/1.73 m²/year), n (%)			
≤ -5	33 (63.5%)	20 (80.0%)	53 (68.8%)
> -5	19 (36.5%)	5 (20.0%)	24 (31.2%)
UPCR stratification (at screening), n (%)			
< 1 gr/gr	41 (78.8%)	21 (84.0%)	62 (80.5%)
≥ 1 gr/gr	11 (21.2%)	4 (16.0%)	15 (19.5%)
UPCR categories at baseline, n (%)			
UPCR < 0.5 gr/gr	36 (69.2%)	20 (80.0%)	56 (72.7%)
0.5 < UPCR < 1 gr/gr	9 (17.3%)	2 (8.0%)	11 (14.3%)
1 ≤ UPCR gr/gr	7 (13.5%)	3 (12.0%)	10 (13.0%)
Treatment with ACEi or ARB, n (%)			
Yes	27 (51.9%)	16 (64.0%)	43 (55.8%)
No	25 (48.1%)	9 (36.0%)	34 (44.2%)

ACEi = Angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; eGFR = estimated glomerular filtration rate; UPCR = urine protein-to-creatinine ratio
1 eGFR slope at screening was based on historical serum creatinine and screening serum creatinine.
2 eGFR slope at baseline was based on historical, screening, and baseline serum creatinine.
Source: Table 14.1.5

Other baseline measures are presented in

Table 15.

Table 15 Other baseline efficacy measures – ITT set

	PRX-102 N=52	Agalsidase Beta N=25	Overall N=77
Height (cm)			
Mean (SE)	170.87 (1.20)	173.20 (1.44)	171.62 (0.94)
SD	8.65	7.21	8.23
Median	170.00	173.00	172.00
Min, Max	148.0; 188.0	162.0; 192.0	148.0; 192.0
Weight (kg)			
Mean (SE)	77.80 (2.38)	81.17 (3.71)	78.89 (2.00)
SD	17.13	18.53	17.54
Median	73.35	79.30	74.40
Min, Max	52.0; 129.0	47.9; 135.0	47.9; 135.0
Region n (%)			
US	33 (63.5%)	18 (72.0%)	51 (66.2%)
ex-US	19 (36.5%)	7 (28.0%)	26 (33.8%)
Duration of the last continuous agalsidase-beta treatment (months) [1, 2]			
n	51	25	76
Mean (SE)	65.54 (6.77)	77.34 (8.25)	69.42 (5.30)
SD	48.31	41.25	46.17
Median	51.55	67.84	57.54
Min, Max	12.6; 236.9	27.6; 168.3	12.6; 236.9
% residual enzyme activity in leukocytes [3]			
Mean (SE)	18.0 (2.52)	25.1 (11.80)	20.3 (4.16)
SD	18.17	58.99	36.49
Median	11.0	8.9	9.7
Min, Max	1.0; 71.9	1.9; 297.0	1.0; 297.0
% residual enzyme activity in plasma [4]			
Mean (SE)	24.0 (4.85)	2526.8 (2477.53)	836.6 (804.62)
SD	35.00	12387.64	7060.55
Median	13.1	3.6	12.4
Min, Max	0.3; 206.1	0.0; 61984.6	0.0; 61984.6
Plasma lyso-Gb3 (nM)			
Mean (SE)	26.22 (3.78)	32.14 (7.08)	28.14 (3.42)
SD	27.27	35.38	30.04
Median	15.20	17.60	17.30
Min, Max	0.8; 143.9	2.1; 142.0	0.8; 143.9
Type of Fabry disease, n (%)			
Classic	27 (51.9%)	14 (56.0%)	41 (53.2%)
Non-classic	25 (48.1%)	11 (44.0%)	36 (46.8%)
Premedication use for agalsidase beta Infusion prior to enrollment, n (%)			
Yes	21 (40.4%)	15 (60.0%)	36 (46.8%)
No	31 (59.6%)	10 (40.0%)	41 (53.2%)
ADA status for PRX-102, n (%) [5]			
Positive	18 (34.6%)	-	-
Negative	34 (65.4%)	-	-
ADA status for agalsidase beta, n (%) [6]			
Positive	-	8 (32.0%)	-
Negative	-	17 (68.0%)	-

ADA = anti-drug antibodies;
1 "Last" treatment refers to patients who had several periods of treatment with agalsidase beta in the past.
2 For one patient in the PRX-102 arm (#33-F20809), due to an error in the reporting in the eCRF, the information on previous treatment with agalsidase beta appeared in the Standard Data Tabulation Model (SDTM) as concomitant treatment rather than as past treatment, and hence this patient was not captured in the table. The subject had been treated with agalsidase beta for more than 11 years (see Listing 16.2.3.7.1). The issue was identified after the interim analysis database lock, and will be corrected for the final database lock.
3 Defined as the value in leukocyte × 100/83.5, where 83.5 nmol/hr/mg protein is the mean between min and max of the lab normal reference range
4 Defined as the value in plasma ×100/12.95, where 12.95 nmol/ hr/mL is the mean of the lab normal reference range

5 The determination of the status is based on the results of the IgG for the assigned drug at baseline.
Source: Table 14.1.5

Fabry Disease Medical History are presented in Table 16.

Table 16 Most common Fabry disease symptoms at study entry - ITT set

Condition	PRX-102 N=52 n (%)	Agalsidase Beta N=25 n (%)	Overall N=77 n (%)
Acroparesthesias	42 (80.8)	19 (76.0)	61 (79.2)
Heat intolerance	39 (75.0)	17 (68.0)	56 (72.7)
Tinnitus	35 (67.3)	17 (68.0)	52 (67.5)
Angiokeratomas	34 (65.4)	17 (68.0)	51 (66.2)
Cornea verticillata	34 (65.4)	15 (60.0)	49 (63.6)
Diarrhea	30 (57.7)	17 (68.0)	47 (61.0)
Abdominal pain	31 (59.6)	13 (52.0)	44 (57.1)
Hypohydrosis	32 (61.5)	12 (48.0)	44 (57.1)
Hearing loss	17 (32.7)	17 (68.0)	34 (44.2)
Headache	23 (44.2)	11 (44.0)	34 (44.2)
Oedema	20 (38.5)	14 (56.0)	34 (44.2)
Vertigo/dizziness	22 (42.3)	9 (36.0)	31 (40.3)
Cardiomyopathy	17 (32.7)	11 (44.0)	28 (36.4)
Arrythmias	17 (32.7)	9 (36.0)	26 (33.8)
Valvular insufficiency	13 (25.0)	10 (40.0)	23 (29.9)
Conduction defects	14 (26.9)	7 (28.0)	21 (27.3)

Source: Table 14.1.6

- Numbers analysed

Of the 78 patients who were randomized, all but one in the pegunigalsidase alfa arm who withdrew consent before receiving any study product were included in the ITT and Safety populations, and all but four in the pegunigalsidase alfa arm who withdrew prior to completing 12 months of treatment were included in the Per Protocol population. Data are presented in Table 17.

Table 17 Number of patients in the analysis populations

Population	PRX-102 N=53	Agalsidase Beta N=25	Overall N=78
ITT	52	25	77
PP	49	25	74
Safety	52	25	77

- Outcomes and estimation

Primary Efficacy Endpoint

Interim Analysis – Non inferiority testing

Data on the primary efficacy endpoint are presented in Table 18.

Table 18 eGFR slope analysis using random intercept random slope (RIRS) model – ITT and PP sets at month 12

	ITT		PP	
Number of subjects:				
PRX-102	52		49	
Agalsidase beta	25		25	
Number of subjects considered in the model:				
PRX-102	52		49	
Agalsidase beta	25		25	
Estimated slopes		95% CI		95% CI
PRX-102	-2.507	-3.835; -1.180	-2.403	-3.714; -1.093
Agalsidase beta	-1.748	-3.585; 0.089	-1.755	-3.566; 0.056
PRX-102 - Agalsidase beta	-0.759	-3.026; 1.507	-0.648	-2.883; 1.587

CI – confidence interval
Notes: Analysis is based on a random intercept random slope longitudinal mixed model with eGFR values at the different time points as dependent variable, treatment arm, time, treatment arm by time interaction and stratification factor (UPCR < 1; ≥ 1 gr/gr) as fixed part of the model. Intercept and slopes are also considered as random effects that vary randomly among patients. An unstructured covariance matrix is considered to model the within-subject correlations, and the Kenward-Roger adjustment is used for the degrees of freedom. The model uses all observations, including unscheduled visits.
Source: Table 14.2.1.4, Table 14.2.1.12

Final Analysis – Non inferiority testing

During the procedure, the applicant submitted the final analysis at month 24 (week 104). As per SAP amendment (see statistical method), the applicant changed its objective from an initial planned superiority testing to a non-inferiority testing).

Data on the primary efficacy endpoint are presented in Table 19 and Table 20 for the ITT and PP sets.

Table 19 Summary of eGFR slopes (first stage of 2-stage ANCOVA and of 2-stage with quantile regression) at week 104

	ITT set		PP set	
	PRX-102 N=52	Agalsidase beta N=25	PRX-102 N=48	Agalsidase beta N=24
eGFR slopes (mL/min/1.73 m^2/year)				
n	51	25	48	24
Mean (SE)	-2.38 (1.25)	-2.31 (0.71)	-2.32 (0.69)	-2.35 (0.74)

SD	8.90	3.56	4.75	3.63
Median (Q1; Q3)	-2.51 (-4.8; 0.8)	-2.16 (-4.6; -0.5)	-2.52 (-4.7; 0.5)	-2.47 (-4.7, -0.4)
Min; Max	-45.3; 28.9	-10.1; 8.1	-16.2; 9.9	-10.1; 8.1

Q1 – 25th percentile; Q3 – 75th percentile
Note: The individual annualized mean change (slope) in eGFR is estimated for each patient with at least 4 eGFR observations using a linear regression model.
Source: Table 14.2.1.5.1, Table 14.2.1.5.2

Table 20 eGFR slope analysis using quantile regression for the median (primary efficacy analysis) – ITT and PP sets at week 104

	ITT		PP	
Number of subjects:				
PRX-102	52		48	
Agalsidase beta	25		24	
Number of subjects considered in the model:				
PRX-102	51		48	
Agalsidase beta	25		24	
Primary model: Estimated median annual eGFR slopes (mL/min/1.73 m^2/year)		95% CI		95% CI
PRX-102	-2.514	-3.788; -1.240	-2.515	-3.666; -1.364
Agalsidase beta	-2.155	-3.805; -0.505	-2.397	-4.337; -0.457
Difference in medians (PRX-102 - Agalsidase beta)	-0.359	-2.444; 1.726	-0.118	-2.450; 2.213

CI – confidence interval; SE – standard error
Notes: Analysis is based on a quantile regression for the median with eGFR slope of each individual patient as dependent variable and treatment arm as covariate of the model. All observations are used including unscheduled visits. Source: Table 14.2.1.4.1, and Table 14.2.1.4

Sensitivity/Supportive Analyses

Sensitivity analyses for Modelling Assumptions- Interim report

Data are presented

Table 21 and Table 22.

Table 21 Summary of eGFR slopes (first stage of 2-stage sensitivity analysis) - ITT set

	PRX-102 N=52		Agalsidase beta N=25	
eGFR slopes (mL/min/1.73 m^2/year)				
n	51		25	
Mean (SE)	-2.55	(1.28)	-1.80	(0.71)
SD	9.15		3.53	
Median	-2.16		-1.77	
Min; Max	-45.3	28.9	-8.1	9.4

Note: The individual annualized mean change (slope) in eGFR is estimated for each patient with at least 4 eGFR observations using a linear regression model and excluding any eGFR values measured during an AKI episode.
Source: Table 14.2.1.5

For the ITT set, the adjusted mean slopes in the ANCOVA were -4.381 for the pegunigalsidase alfa arm and -3.985 for the agalsidase beta arm, the slope difference was -0.396, and the 95% confidence interval was -3.999 to 3.207: i.e., again, the lower limit of the CI was less than -3.0 and hence failed to meet the criterion for non-inferiority. In the equivalent analysis using the PP population, the slope estimates were -3.280 and -2.899 with a difference of -0.382 and a 95% CI of -2.666 to 1.903, so here, non-inferiority was nominally met.

Table 22 Sensitivity analysis: eGFR slope analysis using two-stage ANCOVA – ITT

	ITT		PP	
Number of subjects:				
PRX-102	52		49	
Agalsidase beta	25		25	
Number of subjects considered in the model:				
PRX-102	51		49	
Agalsidase beta	25		25	
Estimated slopes		95% CU		95% CI
PRX-102	-4.381	-6.772; -1.991	-3.280	-4.838; -1.723
Agalsidase beta	-3.985	-7.266; -0.704	-2.899	-4.979; -0.818
PRX-102 - Agalsidase beta	-0.396	-3.999; 3.207	-0.382	-2.666; 1.903

CI = confidence interval
Note: The analysis is based on an ANCOVA model with eGFR slope of each individual patient as dependent variable, treatment arm and stratification factor (UPCR < 1; ≥ 1 g/g) as covariates of the model. All observations are used including unscheduled visits. Source: Table 14.2.1.6.1, Table 14.2.1.6.2

In the analysis using a RI model (similar to the RIRS used for the primary analysis), the slope estimates here were -2.571 and -1.786 for the pegunigalsidase alfa and agalsidase beta arms, respectively. The difference was -0.784 with a 95% CI of -2.803 to 1.234.

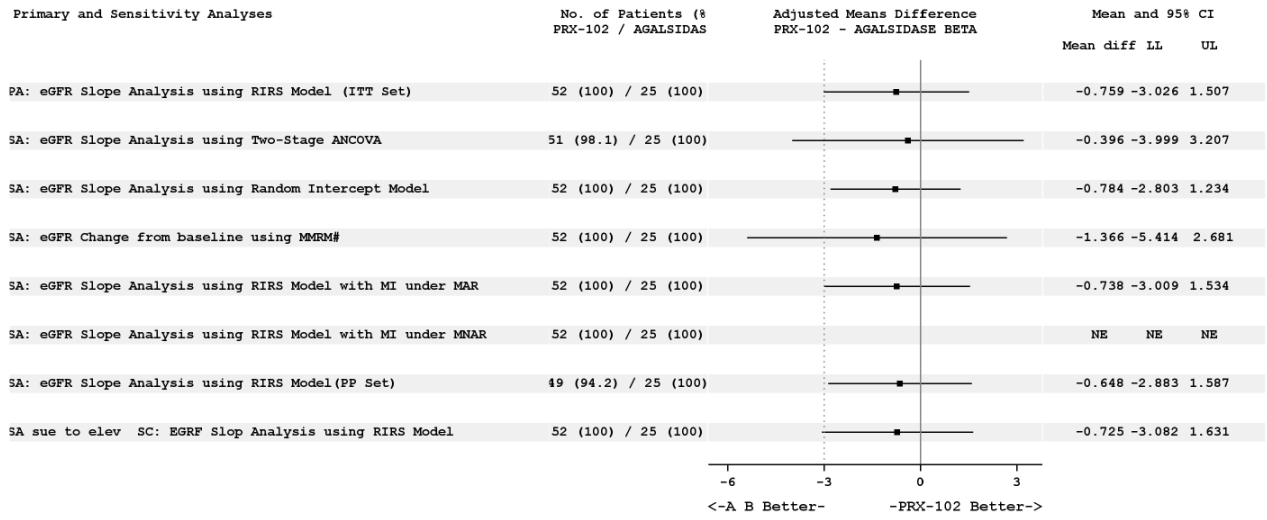
Using an MMRM model, the lower limit of the 95% confidence interval for the difference in eGFR change from baseline between the groups at Week 52 was -5.414, so did not meet the criterion for non-inferiority (Note

that this model does not estimate the slope. Unlike the other models, it does not assume a linear relationship between eGFR and time).

It was specified in the SAP that if any patient experienced an episode of acute kidney injury (AKI), eGFR assessment was not to be done on any samples that were taken during the episode. There was one occurrence of AKI, in a patient in the pegunigalsidase alfa arm. However, this event occurred between sampling visits and resolved before the next sample for eGFR determination was taken; accordingly, there were no exclusions from the analysis for reasons of AKI. To ensure that there was no impact of AKI on the primary endpoint, a sensitivity analysis for AKI identification was done that implemented a criterion based on observed elevated serum creatinine between consecutive visits. The results of this analysis verified that there was no such impact.

The primary model as well as all the sensitivity analyses (excluding the PP in the two-stage model) are summarized in a Forest plot shown in Figure 13.

Figure 13 Forest plot of eGFR difference in slopes, primary and sensitivity analyses - ITT set- at month 12



• PA = Primary Analysis; SA = Sensitivity Analysis; RIRS = Random Intercept Random Slope; MI = Multiple Imputation; MAR = Missing At Random; MNAR = Missing Not At Random; MMRM = Mixed Model Repeated Measures; LL= Lower Limit; UL= Upper Limit; SC=Serum Creatinine
• Vertical dotted line drawn at the prespecified non-inferiority margin for the interim analysis of -3.0 ml/min/1.73 m^2/year #eGFR change from baseline using MMRM evaluated at Week 52

Sensitivity and Supportive Analyses for eGFR annualized slope- Final report

Data are presented in Table 23 and Error! Reference source not found..

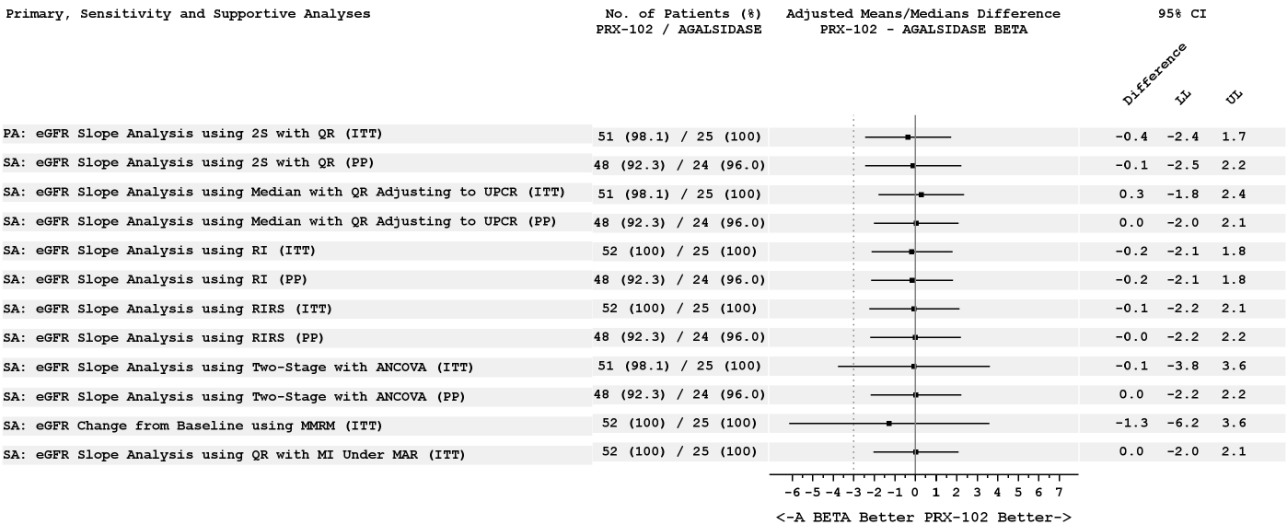
Table 23 Selected sensitivity and supportive analyses for eGFR slope – ITT and PP sets

	ITT		PP	
Number of subjects considered in the models:				
PRX-102	51		48	
Agalsidase beta	25		24	

Sensitivity model: Estimated median annual eGFR slopes (mL/min/1.73 m^2/year) adjusted for UPCR at baseline[1]		95% CI		95% CI
PRX-102	-3.237	-4.934; -1.541	-3.391	-5.129; -1.652
Agalsidase beta	-3.520	-5.502; -1.537	-3.430	-5.549; -1.312
Difference in medians (PRX-102 - Agalsidase beta)	0.282	-1.789; 2.353	0.039	-2.011; 2.090
Sensitivity model (post-hoc): Estimated median annual eGFR slopes (mL/min/1.73 m^2/year) adjusted for gender[1]		95% CI		
PRX-102	-1.984	-3.315; -0.653	-	-
Agalsidase beta	-1.468	-3.212; 0.276	-	-
Difference in medians (PRX-102 - Agalsidase beta)	-0.516	-2.780; 1.748	-	-
Sensitivity model (post-hoc): Estimated median annual eGFR slopes (mL/min/1.73 m^2/year) adjusted for baseline ADA status[1]		95% CI		
PRX-102	-2.388	-3.880; -0.896	-	-
Agalsidase beta	-2.281	-4.131; -0.431	-	-
Difference in medians (PRX-102 - Agalsidase beta)	-0.107	-2.308; 2.093	-	-
Sensitivity model (post-hoc): Estimated median annual eGFR slopes (mL/min/1.73 m2/year) adjusted for FD classification1		95% CI		
PRX-102	-2.059	-3.371; -0.747	-	-
Agalsidase beta	-2.320	-4.113; -0.527	-	-
Difference in medians (PRX-102 - Agalsidase beta)	0.261	-2.020; 2.542	-	-
Supportive analysis: Estimated mean eGFR slopes using random intercept random slope (RIRS) longitudinal mixed model2		95% CI		95% CI
PRX-102	-2.366	-3.641; -1.091	-2.350	-3.623; -1.078
Agalsidase beta	-2.307	-4.088; -0.526	-2.340	-4.137; -0.544
Difference in means (PRX-102 - Agalsidase beta)	-0.059	-2.249; 2.131	-0.010	-2.211; 2.191
Supportive analysis: Estimated mean eGFR slopes using random intercept (RI) longitudinal mixed model3		95% CI		95% CI
PRX-102	-2.467	-3.811; -1.123	-2.474	-3.822; -1.127
Agalsidase beta	-2.294	-3.723; -0.864	-2.310	-3.762; -0.857
Difference in means (PRX-102 - Agalsidase beta)	-0.173	-2.135; 1.789	-0.165	-2.146; 1.817

Analysis was based on a quantile regression for the median with eGFR slope of each individual patient as dependent variable and treatment arm and stratification factors (UPCR<1;≥ 1 g/g or gender) or ADA status, or FD classification (classic/non-classic) as covariates of the model.
RIRS analysis was based on a random intercept random slope longitudinal mixed model with eGFR values at the different time points as dependent variable, treatment arm, time, and treatment arm by time interaction as fixed part of the model. Intercept and slopes was also considered as random effects that vary randomly among patients. An unstructured covariance matrix is considered to model the within subject correlations and the Kenward-Roger adjustment is used for the degrees of freedom.
RI analysis was based on a random intercept longitudinal model with eGFR values at the different time points as dependent variable, treatment arm, time, and treatment arm by time interaction as fixed part of the model. Intercept was also considered as random effect that vary randomly among patients. A compound symmetry covariance matrix was considered to model the within subject correlations and a robust estimate of the covariance matrix was computed using a sandwich estimator.
In all models, all observations were used including unscheduled visits.
Source: Table 14.2.1.4.3, Table 14.2.1.4.3_P1 post-hoc, Table 14.2.1.4.3_P2 post-hoc, Table 14.2.1.4.3_P3 post-hoc, Table 14.2.1.4.4, Table 14.2.1.7.1 to Table 14.2.1.7.4;

Figure 14 Forest plot of eGFR difference in slopes, primary and sensitivity/supportive analyses

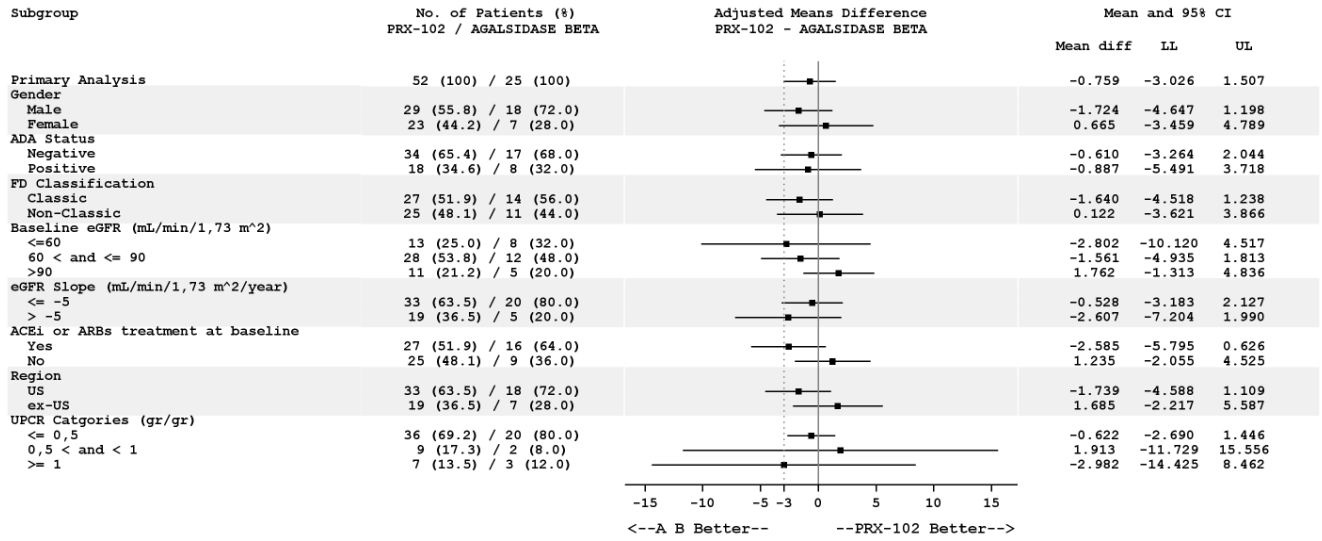


PA = Primary Analysis; SA = Supportive/Sensitivity Analysis; 2S = 2-Stage; QR = Quantile Regression; UPCR = Urine Serum Creatinine Ratio; RI = Random Intercept; RIRS =
Random Intercept Random Slope; ANCOVA = Analysis of Covariance; MI = Multiple Imputation; MAR = Missing at Random; MMRM = Mixed Model Repeated Measures; LL=
Lower Limit; UL= Upper Limit, eGRF = Estimated glomerular filtration rate
Notes:
Vertical dotted line drawn at the prespecified non-inferiority margin of -3.0 ml/min/1.73 m2/year
#eGFR change from baseline using MMRM evaluated at week 104 divided by 2
Source: Figure 15.2.1.4

Subgroup Analyses on eGFR Slope

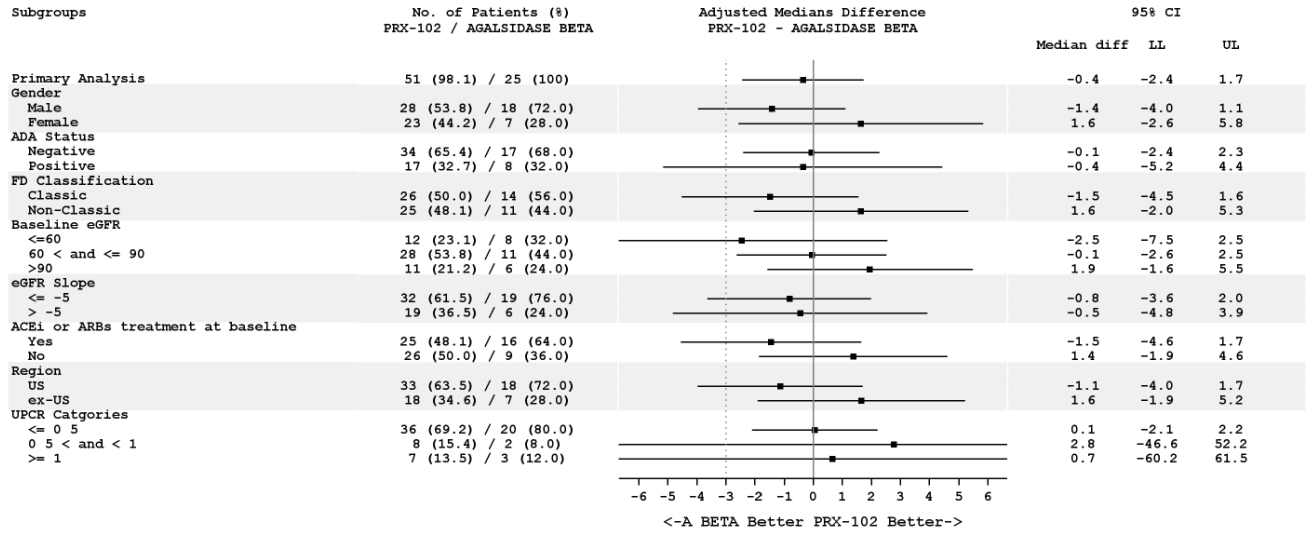
Data are presented in Figure 15 and Figure 16.

Figure 15 Forest plot of eGFR difference in slopes – subgroups- interim report



Notes:
FD = Fabry Disease; UPCR = Urinary protein to creatinine ratio; ACEi = Angiotensin converting enzyme inhibitors; ARBs = Angiotensin receptor blocker; LL= Lower Limit; UL= Upper Limit.
Vertical dotted line drawn at the prespecified non-inferiority margin for the interim analysis of -3.0 ml/min/1.73 m²/year

Figure 16 Forest plot of eGFR difference in slopes – subgroups within ITT set- final report



FD = Fabry Disease; UPCR = Urinary protein to creatinine ratio; ACEi = Angiotensin converting enzyme inhibitors; ARBs = Angiotensin receptor blocker; LL= Lower Limit; UL= Upper Limit, ADA = Anti-drug antibody, eGRF = Estimated glomerular filtration rate
Note: Vertical dotted line drawn at the prespecified non-inferiority margin of -3.0 ml/min/1.73 m²/year

All confidence intervals include 0, but due to the smaller sample size in these subgroups, the CIs are much wider compared to the primary analysis.

Secondary Efficacy Endpoints

- *Renal function*

Data are presented in Table 24.

Table 24 Patients by UPCR categories – ITT set at week 52 and at week 104

		PRX-102 N=52		Agalsidase beta N=25	
Baseline	n	52		25	
	UPCR≤0.5 gr/gr	36	(69.2%)	20	(80.0%)
	0.5 <UPCR <1 gr/gr	9	(17.3%)	2	(8.0%)
	UPCR≥1 gr/gr	7	(13.5%)	3	(12.0%)
Week 52	n	46		24	
	UPCR≤0.5 gr/gr	30	(65.2%)	19	(79.2%)
	0.5 <UPCR <1 gr/gr	9	(19.6%)	2	(8.3%)
	UPCR≥1 gr/gr	7	(15.2%)	3	(12.5%)
Week 104	n	45		24	
	UPCR≤0.5 gr/gr	34	(75.6%)	18	(75.0%)
	0.5 <UPCR <1 gr/gr	5	(11.1%)	2	(8.3%)
	UPCR≥1 gr/gr	6	(13.3%)	4	(16.7%)

Source: Table 14.2.9.1

- *Cardiac function*

Data are presented in Table 24 and Table 26.

Table 25 Summary of left ventricular mass index (g/m²) by hypertrophy status – ITT set- at week 52

	PRX-102 N=52	Agalsidase beta N=25
LVMI for patients with hypertrophy at baseline		
Baseline		
n	12	9
Mean (SE)	115.080 (8.093)	107.761 (8.018)
Change from baseline at Week 52		
n	10	9
Mean (SE)	-2.994 (4.549)	4.767 (4.392)
PRX-102 – agalsidase beta: 95% CI for difference in means	-21.102; 5.580	
LVMI for patients without hypertrophy at baseline		
Baseline		
n	28	13
Mean (SE)	59.218 (2.855)	64.303 (4.751)
Change from baseline at Week 52		
n	23	12
Mean (SE)	0.185 (1.361)	3.669 (2.306)
PRX-102 – agalsidase beta: 95% CI for difference in means	-9.091; 2.123	

Table 26 Summary of left ventricular mass index (g/m2) by gender and hypertrophy status – ITT set- at week 104

	PRX-102		Agalsidase beta	
	Male, N=29	Female, N=23	Male, N=18	Female, N=7
LVMI for patients with hypertrophy at baseline				
Baseline				
n	8	4	7	2
Mean (SE)	111.548 (6.101)	122.145 (22.841)	115.753 (8.436)	81.285 (2.375)
Change from baseline at Week 104				
n	5	4	5	2
Mean (SE)	-2.410 (8.511)	-6.523 (8.557)	5.000 (13.274)	-4.040 (11.090)
PRX-102 – agalsidase beta: 95% CI for difference in means, males: -44.904 ; 30.084				
PRX-102 – agalsidase beta: 95% CI for difference in means, females: -56.257 ; 51.292				
LVMI for patients without hypertrophy at baseline				

Baseline				
n	15	13	8	5
Mean (SE)	67.171 (3.899)	50.021 (2.435)	73.861 (4.005)	49.010 (6.011)
Change from baseline at Week 104				
n	8	11	7	5
Mean (SE)	-1.344 (5.768)	2.820 (3.025)	0.987 (2.740)	-3.682 (4.716)
PRX-102 – agalsidase beta: 95% CI for difference in means, males: -16.573; 11.912				
PRX-102 – agalsidase beta: 95% CI for difference in means, females: -6.582; 19.586				

Source: Table 14.2.6.1.1

It must be noted that the low number of patients in each subgroup limits the interpretation of these data.

Change in Stress Test

Cardiac function was assessed by administering an exercise stress test to evaluate how well the heart handled the demands of increased physical activity. Patients underwent this test at baseline and every 52 weeks.

At week 52: based on those who contributed to data collection (in the pegunigalsidase alfa arm, 29 at baseline and 42 at Week 52; in the agalsidase beta arm, 12 and 21, respectively), the percentage of patients with normal findings increased from 62.1% to 71.4 in the pegunigalsidase alfa arm and from 50.0% to *66.6% in the agalsidase beta arm.*

At week 104: based on those who contributed to data collection (in the pegunigalsidase alfa arm, 29 at baseline and 38 at Week 104; in the agalsidase beta arm, 12 and 17, respectively), the percentage of patients with normal findings decreased from 62.1% to 57.9% in the pegunigalsidase alfa arm and from 50.0% to 64.7% in the agalsidase beta arm.

Changes in Echocardiogram

Patients underwent an echocardiogram at baseline and every 52 weeks for the assessment of aortic, mitral, pulmonic, and tricuspid functions. The majority of patients were normal at all timepoints and for all parameters. However, it is important to note that the echocardiogram procedure was not standardized across sites, so the results are difficult to interpret.

- *Mainz Severity Score Index*

The Mainz Severity Score Index (MSSI) is used to evaluate the severity and progression of clinical signs of Fabry disease, and yields scores for general, neurological, cardiovascular, renal, and overall assessments. An overall score of less than 20 points is considered mild, 20 to 40 is considered moderate, and greater than 40 is considered severe. The MSSI was administered by the Investigator at baseline and every 6 months.

Data are presented in

Table 27 and Table 28.

Table 27 Change in overall score on the Mainz Severity Score Index at Week 52 – ITT set- at week 52

	PRX-102 N=52	Agalsidase beta N=25
Baseline		
n	49	25
Mean (SE)	23.16 (1.42)	25.12 (2.13)
Week 52		
n	45	23
Mean (SE)	23.38 (1.68)	24.09 (2.19)
Change from baseline at Week 52		
Mean (SE)	-0.47 (0.66)	-0.91 (1.09)
95% CI for the change from baseline	-1.80; 0.87	-3.18; 1.36
PRX-102 – agalsidase beta: 95% CI for difference in means	-2.1; 3.0	

Note: If the confidence interval does not contain 0 that suggests a statistically significant difference between the treatment groups.
Source: Table 14.2.11.1

Table 28 Change in overall score on the Mainz Severity Score Index at Week 104 – ITT set at week 104

	PRX-102 N=52	Agalsidase beta N=25
Baseline		
n	49	25
Mean (SE)	23.18 (1.42)	25.16 (2.14)
Week 104		
n	46	23
Mean (SE)	22.11 (1.80)	27.09 (2.30)
Change from baseline at Week 104		
Mean (SE)	-2.07 (0.77)	2.04 (1.10)
95% CI for the change from baseline	-3.62; -0.52	-0.24; 4.33
PRX-102 – agalsidase beta: Difference in means (95% CI)	-4.11 (-6.8; -1.4)	

Note If the confidence interval does not contain 0, that suggests a statistically significant difference between the treatment groups.

- *Pain*

Change in Frequency of Use of Pain Medication

At least one pain medication was taken at any time during the study by 37 (71.2%) of patients in the pegunigalsidase alfa arm and by 22 (88.0%) in the agalsidase beta arm. The most common medications were

paracetamol and ibuprofen. In addition, 1 patient [4.0%] in the agalsidase beta arm took a topical form of ibuprofen. Patients were broadly categorized as taking no medications, taking one, or taking two or more.

Data are presented in Table 29 and Table 30.

Table 29 Number of patients with pain medication use, shift from baseline to last visit – ITT set week 52

	Pain Medication Use at Baseline															
	PRX-102 (N = 52)								Agalsidase beta (N = 25)							
Pain medication use at last visit	0 (n=23)		1 (n=18)		2+ (n=11)		Overall (n=52)		0 (n=8)		1 (n=9)		2+ (n=8)		Overall (n=25)	
0	20	(87.0%)	2	(11.1%)	1	(9.1%)	23	(44.2%)	7	(87.5%)	2	(22.2%)	0		9	(36.0%)
1	2	(8.7%)	16	(88.9%)	0		18	(34.6%)	1	(12.5%)	7	(77.8%)	0		8	(32.0%)
2+	1	(4.3%)	0		10	(90.9%)	11	(21.2%)	0		0		8	(100.0%)	8	(32.0%)
Overall	23	(100.0%)	18	(100.0%)	11	(100.0%)	52	(100.0%)	8	(100.0%)	9	(100.0%)	8	(100.0%)	25	(100.0%)

0-2+ categories refer to the number of pain medications taken by patients. Pain medication counting is done based on Standardized Medication Name.
Source: Table 14.2.13.3

Table 30 Number of patients with pain medication use, shift from baseline to last visit – ITT set week 104

	Pain Medication Use at Baseline															
	PRX-102 (N = 52)								Agalsidase beta (N = 25)							
Pain medication use at last visit	0 (n=23)		1 (n=17)		2+ (n=12)		Overall (n=52)		0 (n=8)		1 (n=9)		2+ (n=8)		Overall (n=25)	
0	20	(87.0%)	2	(11.1%)	1	(8.3%)	23	(44.2%)	8	(100.0%)	2	(22.2%)	0		10	(40.0%)
1	2	(8.7%)	15	(88.2%)	0		17	(32.7%)	0		7	(77.8%)	0		7	(28.0%)
2+	1	(4.3%)	0		11	(91.7%)	12	(23.1%)	0		0		8	(100.0%)	8	(32.0%)
Overall	23	(100.0%)	17	(100.0%)	12	(100.0%)	52	(100.0%)	8	(100.0%)	9	(100.0%)	8	(100.0%)	25	(100.0%)

0-2+ categories refer to the number of pain medications taken by patients. Pain medication counting is done based on Standardized Medication Name.
Note, percentages were calculated based on n in each category
Source: Table 14.2.13.3

Change in Severity of Pain

The Short Form Brief Pain Inventory (BPI) is designed to rapidly assess the severity of pain and its impact on functioning, and yields scores for “Pain at Its Worst in Last 24 Hours”, “Pain at Its Least in Last 24 Hours”, “Pain Right Now”, and “Pain on Average”. The scales are scored from 1 to 10, with a score of 1–4 points indicating mild pain, 5–6 indicating moderate, and 7–10 indicating severe. The BPI was administered at baseline and every 6 months.

Table 31 shows the change from baseline for the 2 scores “Pain at Its Worst in Last 24 Hours” and “Pain on Average”at interim analysis (Week 52) and Table 32 shows the change from baseline at interim analysis (Week 104).

Table 31 Change in scores on Short Form Brief Pain Inventory at Week 52 – ITT set

Pain Severity	PRX-102 N=52	Agalsidase beta N=25
Pain at its Worst in Last 24 Hours		
Baseline		
n	52	25
Mean (SE)	3.5 (0.4)	2.6 (0.6)
Change from baseline at Week 52		
n	45	23
Mean (SE)	0.0 (0.4)	-0.1 (0.2)
95% CI for the change from baseline	-0.7; 0.7	-0.5; 0.3
PRX-102 – agalsidase beta: 95% CI for difference in means	-0.7; 0.9	
Pain on Average		
Baseline		
n	52	25
Mean (SE)	2.2 (0.3)	2.2 (0.4)
Change from baseline at Week 52		
n	45	23
Mean (SE)	0.2 (0.2)	0.0 (0.3)
95% CI for the change from baseline	-0.3; 0.7	-0.7; 0.8
PRX-102 – agalsidase beta: 95% CI for difference in means	-0.7; 1.0	

Note: If the confidence interval does not contain 0 that suggests a statistically significant difference between the treatment groups.
Source: Table 14.2.8.1

Table 32 Change in scores on Short Form Brief Pain Inventory at Week 104 – ITT set

Pain Severity	PRX-102 N=52	Agalsidase beta N=25
Pain at its Worst in Last 24 Hours		
Baseline		
n	52	25
Mean (SE)	3.5 (0.4)	2.6 (0.6)
Change from baseline at Week 104		
n	45	22
Mean (SE)	-0.1 (0.5)	0.6 (0.6)
95% CI for the change from baseline	-1.1 ; 0.8	-0.7 ; 1.8
PRX-102 – agalsidase beta: Difference in means (95% CI)	-0.7 (-2.2 ; 0.8)	
Pain on Average		
Baseline		
n	52	25

Mean (SE)	2.2 (0.3)	2.2 (0.4)
Change from baseline at Week 104		
n	45	22
Mean (SE)	0.4 (0.3)	0.2 (0.4)
95% CI for the change from baseline	-0.3 ; 1.0	-0.6 ; 1.0
PRX-102 – agalsidase beta: Difference in means (95% CI)	0.2 (-0.9 ; 1.2)	

Note: If the confidence interval does not contain 0, that suggests a statistically significant difference between the treatment groups.

- *Change in Quality of Life Scores*

The Quality of Life questionnaire (EQ-5D-5L) covers five domains: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression. For each domain, respondents select one of five options ranging from no problem in performing a function to inability to perform the function, or ranging from no problem to an extreme problem, as applicable. The EQ-5D-5L was administered at baseline and every 6 months.

Data are presented in Table 33 and Table 34.

Table 33 Proportion of patients with improvement or no change in quality of life assessments at Week 52 - ITT set

		PRX-102 N=52		Agalsidase beta N=25	
Number of patients with data at Week 52		n=45		n=23	
Mobility	Improvement or no change	37	(82.2%)	20	(87.0%)
	Worsening	8	(17.8%)	3	(13.0%)
Self-care	Improvement or no change	41	(91.1%)	22	(95.7%)
	Worsening	4	(8.9%)	1	(4.3%)
Usual activities	Improvement or no change	34	(75.6%)	21	(91.3%)
	Worsening	11	(24.4%)	2	(8.7%)
Pain/Discomfort	Improvement or no change	35	(77.8%)	19	(82.6%)
	Worsening	10	(22.2%)	4	(17.4%)
Anxiety/Depression	Improvement or no change	37	(82.2%)	21	(91.3%)
	Worsening	8	(17.8%)	2	(8.7%)

Source: Table 14.2.12.3

Table 34 Proportion of patients with changes in quality of life assessments at Week 104 - ITT set

		PRX-102 N=52		Agalsidase beta N=25	
Number of patients with data at Week 104		n=46		n=22	
Mobility	Improvement or no change	41	(89.1%)	19	(86.4%)
	Worsening	5	(10.9%)	3	(13.6%)
Self-care	Improvement or no change	41	(89.1%)	20	(90.9%)

	Worsening	5	(10.9%)	2	(9.1%)
Usual activities	Improvement or no change	36	(78.3%)	20	(90.9%)
	Worsening	10	(21.7%)	2	(9.1%)
Pain/Discomfort	Improvement or no change	38	(82.6%)	16	(72.7%)
	Worsening	8	(17.4%)	6	(27.3%)
Anxiety/Depression	Improvement or no change	39	(84.8%)	20	(90.9%)
	Worsening	7	(15.2%)	2	(9.1%)

- *Fabry Clinical Events*

Data are presented in Table 35 and Table 36.

Table 35 Number of patients with Fabry clinical events - Safety set week 52

	PRX-102		Agalsidase beta	
Fabry clinical events categories	Number of Patients N=52	Number of Events	Number of Patients N=25	Number of Events
Overall	8 (15.4%)	10	1 (4.0%)	1
Renal events	1 (1.9%)	1	0	0
Cardiac events	5 (9.6%)	6	1 (4.0%)	1
Cerebrovascular events	3 (5.8%)	3	0	0
Non-cardiac related death	0	0	0	0

Source: Table 14.2.14

Table 36 Number of patients with Fabry clinical events - ITT set Week 52

	PRX-102		Agalsidase beta	
Fabry clinical events categories	Number (%) of Patients N=52	Number of Events (Rate)	Number (%) of Patients N=25	Number of Events (Rate)
Overall	9 (17.3%)	11 (11.2)	2 (8.0%)	2 (4.0)
Cardiac events	6 (11.5%)	7 (7.1)	2 (8.0%)	2 (4.0)
Cerebrovascular events	3 (5.8%)	3 (3.1)	0	0
Renal events	1 (1.9%)	1 (1.0)	0	0

Non-cardiac related death	0	0	0	0

- *Biomarkers of Fabry disease*

Plasma lyso-Gb3

Blood samples were taken at baseline, Week 6, every 3 months up to a year, and then every 6 months up to 2 years.

Data are presented in Table 37 and Table 38.

Table 37 Change from baseline of plasma lyso-Gb3 concentrations at Week 52 – ITT set

	PRX-102 N=52	Agalsidase beta N=25
Plasma Lyso-Gb3 Concentration (nM)		
Baseline		
n	52	25
Mean (SE)	26.22 (3.78)	32.14 (7.08)
Change from baseline at Week 52 (nM)		
n	47	24
Mean (SE)	2.21 (1.12)	-7.92 (4.51)
Percent (%) change from baseline at Week 52		
Mean (SE)	10.28 (3.32)	-10.43 (4.38)

Source: Table 14.2.3.1

Table 38 Change from baseline of plasma lyso-Gb3 concentrations at Week 104 – ITT set

	PRX-102 N=52	Agalsidase beta N=25
Plasma Lyso-Gb3 Concentration (nM)		
Baseline		
n	52	25
Mean (SE)	26.22 (3.78)	32.14 (7.08)
Change from baseline at Week 104 (nM)		
n	46	22
Mean (SE)	3.30 (1.38)	-8.74 (4.85)
Percent (%) change from baseline at Week 104		
Mean (SE)	10.34 (3.80)	-12.69 (4.60)

Source: Table 14.2.3.1

Lyso-Gb3 in urine

Levels of lyso-Gb3 in urine were assessed on the same schedule as for plasma lyso-Gb3. Data are presented in Table 39 and Table 40.

Table 39 Change from baseline in urine lyso-gb3 concentrations at Week 52 - ITT set

	PRX-102 N=52	Agalsidase beta n=25
Urine Lyso-Gb3 Concentration (pM/mM creatinine)		
Baseline		
n	48	22
Mean (SE)	48.1 (7.8)	44.5 (10.9)
Change from baseline at Week 52 (pM/mM creatinine)		
n	38	19
Mean (SE)	3.3 (4.5)	-16.0 (5.1)
PRX-102 – agalsidase beta: 95% CI for difference in means	5.7; 33.0	
Percent (%) change from baseline at Week 52		
Mean (SE)	28.65 (13.28)	-18.84 (9.21)
PRX-102 – agalsidase beta: 95% CI for difference in means	15.1; 79.9	

Note: If the confidence interval does not contain 0 that suggests a statistically significant difference between the treatment groups.
Source: Table 14.2.5.1

Table 40 Change from baseline in urine lyso-Gb3 concentrations at Week 104 - ITT set

	PRX-102 N=52	Agalsidase beta n=25
Urine Lyso-Gb3 Concentration (pM/mM creatinine)		
Baseline		
n	48	22
Mean (SE)	48.1 (7.8)	44.5 (10.9)
Change from baseline at Week 104 (pM/mM creatinine)		
n	37	19
Mean (SE)	7.0 (7.7)	-11.2 (4.7)
PRX-102 – agalsidase beta: Difference in means (95% CI)	18.1 (0.1;36.1)	
Percent (%) change from baseline at Week 104		
Mean (SE)	33.00 (13.19)	-16.14 (9.72)
PRX-102 – agalsidase beta: Difference in means (95% CI)	49.1 (16.3;82.0)	

The confidence intervals at both post-baseline time points did not contain 0, which suggests a difference in favour of agalsidase beta.

Gb3 in plasma

Levels of Gb3 in plasma were assessed on the same schedule as plasma lyso-Gb3. Data are presented in Table 41 and Table 42.

Table 41 Change from baseline in plasma Gb3 concentrations at Week 52 – ITT set

	PRX-102 N=52	Agalsidase beta N=25
Plasma Gb3 Concentration (nM)		
Baseline		
n	52	25
Mean (SE)	5087.7 (282.9)	4695.4 (499.9)
Change from baseline at Week 52 (nM)		
n	45	23
Mean (SE)	121.6 (249.2)	-368.9 (396.2)
PRX-102 – agalsidase beta: 95% CI for difference in means	-455.7; 1436.6	
Percent (%) change from baseline at Week 52		
Mean (SE)	5.50 (5.75)	-2.25 (4.80)
PRX-102 – agalsidase beta: 95% CI for difference in means	-7.2;22.7	

Note: If the confidence interval does not contain 0 that suggests a statistically significant difference between the treatment groups. Source: Table 14.2.4.1

Table 42 Change from baseline in plasma Gb3 concentrations at Week 104 – ITT set

	PRX-102 N=52	Agalsidase beta N=25
Plasma Gb3 Concentration (nM)		
Baseline		
n	52	25
Mean (SE)	5087.7 (282.9)	4695.4 (499.9)
Change from baseline at Week 104 (nM)		
n	46	22
Mean (SE)	138.0 (214.4)	-81.8 (314.7)
PRX-102 – agalsidase beta: Difference in means (95% CI)	219.8 (-549.3;988.9)	
Percent (%) change from baseline at Week 104		
Mean (SE)	4.59 (4.48)	2.69 (4.36)
PRX-102 – agalsidase beta: Difference in means (95% CI)	1.9 (-10.6;14.4)	

As the confidence intervals contained 0 in each case, this suggests no difference between the two arms.

- Ancillary analyses

Not applicable

- Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 43 Summary of efficacy for trial PB-102-F20

Title: *Protocol PB-102-F20 Title: A Randomized, Double-blind, Active Control Study of the Safety and Efficacy of PRX-102 Compared to Agalsidase Beta on Renal Function in Patients with Fabry Disease Previously Treated with Agalsidase Beta*		
Study identifier	*PB-102-F20* EudraCT number: 2016-000378-38 NCT Number: NCT02795676	
Design	Study PB-102-F20 study is a randomized, double-blind, active control study of the safety and efficacy of Pegunigalsidase alfa (PRX-102) compared to agalsidase beta (Fabrazyme) on renal function in patients with Fabry disease previously treated with agalsidase beta. An Interim Analysis occurred when the last patient completed the 12-month visit and uses to support a Marketing Authorization Application (MAA) submission in the EU. The final analysis will occur when the last patient completes the 24-month visit and will support a submission to the another regulatory authority.	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	24 months (with 12 months interim analysis) Not applicable 60 months
Hypothesis	Non-inferiority	
Treatments groups	Pegunigalsidase alfa (PRX-102)	Pegunigalsidase alfa (PRX-102) 1 mg/kg, intravenously over 3 hours, every 2 weeks. After the first 3 months, infusion time could be reduced gradually to 1.5 hours pending patient tolerability, PI evaluation, and Medical Monitor approval.

	Agalsidase beta (Fabrazyme)		Agalsidase beta 1 mg/kg, intravenously over 3 hours, every 2 weeks. After the first 3 months, infusion time could be reduced gradually to 1.5 hours pending patient tolerability, PI evaluation, and Medical Monitor approval.
Endpoints and definitions	Primary endpoint	eGFR Slope (mL/min/1.73 m^2/year)	The annualised change in eGFR. Comparison of annualised mean change (slope) for eGFR between the pegunigalsidase alfa group and the agalsidase beta (Control) Interim analysis done using a random intercept random slope longitudinal mixed model. All available eGFR data were used, i.e. also data beyond 12 months. For the final analysis the first stage includes estimation of individual slopes using linear regression. Two models were considered for the 2nd stage: Analysis of Covariance (ANCOVA) and quantile regression for comparing the median slopes Non-inferiority was to be declared if the lower bound of the 95% CI for the interaction term of treatment groups (ITT population) by time was greater than -3.0 mL/min/1.73 m^2/year, the prespecified non-inferiority margin.
	Secondary endpoint	eGFR (mL/min/1.73 m^2)	Kidney function (estimated glomerular filtration rate [eGFR] was calculated based on measured serum creatinine levels according to CKD-EPI formula. Descriptive statistics of eGFR absolute values and change from Baseline.
	Secondary endpoint	Plasma Lyso-Gb3(ng/mL)	Descriptive statistics of Globotriaosylsphingosine (Lyso-Gb3) absolute values and absolute change of Lyso-Gb3 concentrations in plasma from baseline to month 60.
	Secondary endpoint	Short Form Brief Pain Inventory (BPI)	Descriptive statistics of the pain severity, and pain interference [(for each item and for the composite (mean) severity of interference)].
	Secondary endpoint	Mainz Severity Score Index (MSSI)	Descriptive statistics of the qualitative assessments regarding the sign/symptom in general, neurological, cardiovascular, renal dysfunction and the total change in each score.
	Secondary endpoint	Fabry Clinical Events (FCEs)	Descriptive statistics of number of events, assessed throughout studies
Database lock	Database lock for Interim Analysis occurred on19 Apr 2021 Database lock for final Analysis occurred on 16 March 2022		

Results and Analysis			
Analysis description	Primary Analysis		
Analysis population and time point description	Intent to treat		
Notes	Interim Analysis was conducted when the last patient completed the 12-month visit. All available eGFR data were used for the primary analysis (i.e., also data beyond 12 months).		
		pegunigalsidase alfa (overall)	Agalsidase beta (Fabrazyme)
	Number of subjects	52	25
Descriptive statistics and estimate variability	eGFR Slope (mL/min/1.73 m^2/year); LSMean Baseline mean(SE) Month 12 LS Mean(95%CI) Month 24 median (95%CI)	 -8.07 (0.91) -2.507 (-3.835; -1.180) -2.514 (-3.788; -1.240)	 -8.48 (0.83) -1.748 (-3.585; 0.089) -2.155 (-3.805; -0.505)
Effect estimate per comparison	Primary endpoint eGFR slopes Interim analysis using a random intercept random slope longitudinal mixed model	Comparison groups	pegunigalsidase alfa vs Agalsidase beta
		Difference between groups in LS Means	-0.759
		variability statistic (confidence interval)	(-3.026; 1.507)
		p-value	0.506
	Final analysis. eGFR slope analysis using quantile regression for the median	Difference in medians (pegunigalsidase alfa - Agalsidase beta)	-0.359
		variability statistic (confidence interval)	-2.444; 1.726
Notes			
Analysis description	Secondary Efficacy Endpoints		
Analysis population and time point description	Intent to treat		
Descriptive statistics and estimate variability		pegunigalsidase alfa (overall)	Agalsidase beta (Fabrazyme)
	Number of subjects	52	25

	eGFR (mL/min/1.73 m²) ; media (SE) Baseline Month 12 Change from BL	 73.25 (2.75) 73.81 (3.01) -0.47 (1.10)	 73.49 (4.04) 74.42 (4.51) 0.93 (1.81)
	plasma Lyso-Gb3(nM); Mean (SE) Baseline Month 12 Change from BL Month 24 Change from BL Month 24	 26.22 (3.78) 28.12 (4.03) 2.21 (1.12) 29.22 (4.48) 3.30 (1.38)	 32.14 (7.08) 24.98 (4.69) -7.92 (4.51) 19.65 (3.60) -8.74 (4.85)
	BPI, average pain severity score; Mean (SE) Baseline Month 12 Change from BL Month 24 Change from BL	 2.2 (0.3) 2.3 (0.3) 0.2 (0.2) 2.6 (0.4) 0.4 (0.3)	 2.2 (0.4) 2.2 (0.4) 0.0 (0.3) 2.5 (0.5) 0.2 (0.4)
	MSSI overall score; Mean (SE) Baseline Month 12 Change from BL Month 24 Change from baseline	 23.16 (1.42) 23.38 (1.68) -0.47 (0.66) 22.11 (1.80) -2.07 (0.77)	 25.12 (2.13) 24.09 (2.19) -0.91 (1.09) 27.09 (2.30) 2.04 (1.10)
	FCE; n (%)Month 12 FCE; n (%)Month 24	8 (15.4%) 9 (17.3%)	1 (4.0%) 2 (8.0%)
Notes	A total of 78 patients were randomised (2:1) and 77 patients (PRX-102: 52; Fabrazyme: 25) were treated. For the interim analysis, all but 3 patients completed the 12 month interim treatment period.		

2.6.5.3. Clinical studies in special populations

No clinical studies targeting elderly or patients with renal or hepatic impairment were performed.

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Although there was no formal pooling of study data, additional efficacy analyses across trials on the main efficacy outcomes that were assessed in all studies were submitted during the procedure. This in order to support the assessment of the totality of the evidence.

- *Renal function parameter*

Data are presented in Table 44 and

Table 45.

Table 44 Comparison of Kidney Function Data for Pegunigalsidase Alfa 1 mg/kg EOW Across Studies and Populations, (Mean (SE) [Median]), Efficacy Populations

Parameter		PB-102-F01/02/03 ERT-naïve (N=15)		PB-102-F20 Fabrazyme-experienced N = 52		PB-102-F30 Replagal-experienced (N=20)	
eGFR (ml/min/1.73 m^2)		n		n		n	
	Baseline	15	111.7 (5.5) [114.3]	52	73.46 (2.80) [73.45]	20	79.46 (4.92) [82.18]
	End of Study[a]	11	106.9 (5.5) [112.2]	47	70.53 (3.19) [69.35]	20	76.91 (5.22) [77.43]
	Change from Baseline	11	-0.4 (1.3) [-1.0]	47	-3.60 (1.58) [-2.39]	20	-2.56 (2.14) [-3.39]
eGFR slope (ml/min/1.73 m^2/yr)	Baseline	-	Not available	52	-8.03 (0.92) [-6.70] Range: -30.5 ; 6.3	20	-5.90 (1.34) [-4.41] Range: -20.5 ; 4.8
	End of Study[a]	15	-1.6 (0.8) [-1.5] Range: -6.5 ; 4.9	51	-2.38 (1.25) [-2.51] Range: (-45.3; 28.9)	20	-1.19 (1.77) [-0.72] Range: -18.6; 14.2
Proportion of patients reaching kidney therapeutic goal[b]	End of Study[a]	-	Not available	52	41 patients (80.4%)	20	13 patients (65.0%)

a. Month 12 for study ,PB-102-F30; Month 24 for studies PB-102-F01/02/03 -F20
b. Based on eGFR slope categories according to Wanner 2018)

Table 45 UPCR Category under Treatment with 1 mg/kg EOW Pegunigalsidase alfa Across Studies and Dosing Regimens – Efficacy Populations

	PB-102F01/02/03 ERT-naïve	PB-102-F20 Fabrazyme experienced	PB-102-F30 Replagal experienced
Baseline			
n	15	52	20
Normal to mildly increased, n (%)	10 (66.7)	36 (69.2)	13[a] (65.0)
Moderately increased, n (%)	5 (33.3)	9 (17.3)	3 (15.0)
Severely increased, n (%)	0 (0.0)	7 (13.5)	4 (20.0)
End of Study			
n	11	45	20
Normal to mildly increased, n (%)	6 (54.5)	34 (75.6)	13[a] (65.0)
Moderately increased, n (%)	5 (45.5)	5 (11.1)	2 (10.0)
Severely increased, n (%)	0 (0.0)	6 (13.3)	5 (25.0)

KDIGO = Kidney Disease: Improving Global Outcomes; n = number of patients with data; N = number of patients overall; UPCR = urine protein to creatinine ratio; V = visit.
UPCR category based on the KDIGO guidelines (units g/g) as follows: normal to mildly increased (UPCR < 0.15); moderately increased (0.15 ≤ UPCR ≤ 0.5); severely increased (0.5 > UPCR).
a. 10 and 9 patients had "protein undetectable" at Baseline and Month 12, respectively. These patients were included in the category: normal.
Reference: Efficacy results sections in individual study reports

- *Cardiac parameter: Left ventricular mass index (LVMI)*

Data are presented in Table 46.

Table 46 Left Ventricular Mass Index (g/m²) Across Studies, by Gender and Overall

Study Time point	Male Patients	Female Patients	Overall
PB-102-F01/02/03			
Baseline n Mean (SE)	n=8 63.3 (3.1)	n=7 40.7 (3.3)	n=15 52.7 (3.7)
Month 24 n Mean (SE)	n=7 63.2 (1.5)	n=4 50.8 (6.7)	n=11 58.7 (3.1)
Mean difference [g/m²] n Mean (SE)	n=7 -2.7 (1.9)	n=4 10.3 (4.9)	n=15 2.0 (2.8)
PB-102-F20			
Baseline n Mean (SE)	n=23 82.61 (5.54)	n=17 66.99 (9.22)	n=40 75.97 (5.13)
Month 24 n Mean (SE)	n=15 82.03 (7.04)	n=20 63.72 (7.04)	n=35 71.56 (5.20)
Mean difference [g/m²] n Mean (SE)	n=13 -1.75 (4.61)	n=15 0.33 (3.19)	n=28 -0.64 (2.69)
PB-102-F30			
Baseline n Mean (SE)	n=13 97.6 (8.9)	n=7 66.9 (5.8)	n=20 86.9 (6.9)

Month 12 n Mean (SE)	n=12 98.3 (7.8)	n=7 74.1 (5.0)	n=19 89.4 (6.1)
Mean difference [g/m^2] n Mean (SE)	n=12 2.4 (3.4)	n=7 7.1 (5.0)	n=19 4.1 (2.8)

- Reference: PB-102-F30 CSR, Table 14.2.2.5.1; PB-102-F03 CSR, Table 14.2.7.1.1; PB-102-F20 post hoc Table REQ04_14.2.6.1a and Table REQ04_14.2.6.1.1a.

- *Signs and symptoms according to the Mainz Severity Score Index and average pain severity*

Data are presented in Table 47.

Table 47 Evaluation of Fabry disease severity of Symptoms and pain Across Studies

	PB-102-F01/02/03 ERT-naïve (N=15)	PB-102-F20 Non-naïve (N = 52)	PB-102-F30 Non-naïve (N=20)
MSSI, overall score[a]; [n] Mean (SE)			
Baseline	[15] 21.5 (2.5)	[49] 23.18 (1.42)	[20] 20.3 (2.2)
End of Study[b]	[11] 13.3 (2.7)	[46] 22.11 (1.80)	[20] 19.3 (2.4)
Change from Baseline	[11] -7.5 (1.8)	[46] -2.07 (0.77)	[20] -1.0 (0.9)
BPI, average pain severity score[a]; [n] Mean (SE)			
Baseline	[15] 3.7 (0.7)	[52] 2.2 (0.3)	[20] 1.9 (0.4)
End of Study[b]	[11] 3.0 (0.8)	[45] 2.6 (0.4)	[20] 1.9 (0.5)
Change from Baseline	[11] -0.5 (0.8)	[45] 0.4 (0.3)	[20] 0.1 (0.2)

- Note when changes from baseline do not reflect differences between the presented values at baseline and Month 12/24, this is due to different sample sizes at the timepoints a. Higher numbers for the scores indicate higher intensity/severity
- b. Month 12 for study, PB-102-F30; Month 24 for studies PB-102-F01/02/03 and -F20

- *Integrated analysis of annualised eGFR slope*

An integrated analysis of annualised eGFR slope has been generated including data across the development program of 1 mg/kg EOW (PB-102-F03, PB-102-F30, PB-102-F20, and PB-102-F60), compared with originally naïve patients (PB-102-F03), and across all studies (PB-102F03, PB-102-F30, PB-102-F20, PB-102-F50, PB-102-F51 and PB-102-F60), with both the tested dose regimens. Only patients with at least 18 months of exposure were included.

The integrated analysis covers treatment of up to 7.6 years with 1 mg/kg EOW (and up to 3.75 years for 2 mg/kg E4W).

Table 48 Annualized eGFR Slope (mL/min/1.73 m2/year) under Treatment with Pegunigalsidase alfa - Integrated Efficacy Population[a]

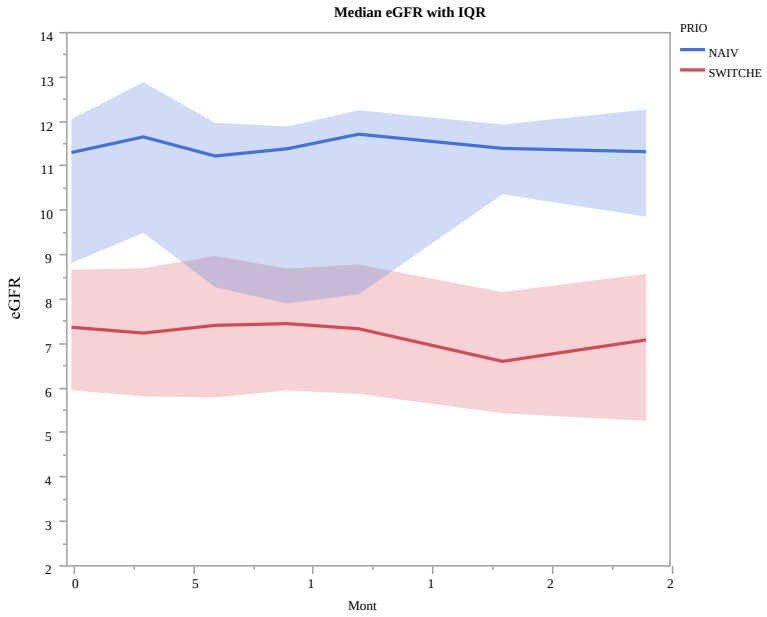
	Naïve N=11	Dosing: 1 mg/kg EOW N=84	Any dosing N=113
Mean (SE)	-2.56 (0.36)	-2.80 (0.44)	-2.80 (0.35)
Median (min; max)	-2.24 (-4.5;-1.3)	-2.62 (-19.4; 9.9)	-2.62 (-19.4; 9.9)

eGFR = estimated glomerular filtration rate; EOW = every other week; N: Number of patients in cohort; max = maximum; min = minimum; SE = standard error

a. The integrated analyses include data across the development program of 1 mg/kg EOW (PB-102-F03, PB-102-F30, PB-102-F20, and PB-102-F60), compared with originally naïve patients (PB-102-F03), and across all studies (any dosing; PB-102-F03, PB-102-F30, PB-102-F20, PB-102-F50. PB-102-F51 and PB-102-F60). Reference: ISE Tables 6.1 and 6.2.

Effect in kidney function during long-term treatment with pegunigalsidase alfa 1 mg/kg EOW up to 24 months (after which sample size decreased) is visualised in Figure 17.

Figure 17 Median (IQR) eGFR (mL/min/1.73 m²) Values over Time with Pegunigalsidase alfa 1 mg/kg EOW Treatment in ERT-naïve[a] and ERT-experienced[a] (Integrated Analysis) Patients



IQR = inter quartile range

a. Patients in studies PB-102-F01/F02/F03 (naïve) or PB-102-F20, PB-102-F30, PB-102-F60 (experienced) having received pegunigalsidase alfa 1 mg/kg EOW for at least 18 months.

Reference: ISE Tables 6.1 and 6.2.

2.6.5.6. Supportive study(ies)

In addition to the main study, 3 supportive Phase 3 studies were submitted to support this application:

- PB-102-F30 included patients switching to treatment with 1.0 mg/kg EOW pegunigalsidase alfa after treatment with agalsidase alfa (Replagal) for at least 2 years;
- PB-102-F50 investigating the alternative dose of 2.0 mg/kg E4W in ERT pre-treated patients;

- PB-102-F51 Open Label Extension Study to Evaluate the Long-Term Safety and Efficacy of pegunigalsidase alfa 2 mg/kg E4W (interim analysis).

Study PB102-F30

Study PB-102-F30, BRIDGE, was an open-label, switch-over study to assess the safety and efficacy of pegunigalsidase alfa in adult patients with Fabry disease previously treated with agalsidase alfa (Replagal).

Patients were screened and evaluated over 3 months while continuing on agalsidase alfa and eligible patients were switched from agalsidase alfa to receive IV infusions of pegunigalsidase alfa at 1 mg/kg EOW over a 12-month duration.

Study participants were aged between 18-60 years, male or female with documented diagnosis of Fabry disease; eGFR ≥40 mL/min/1.73 m² by CKD-EPI equation, and treatment with agalsidase alfa for at least 2 years and on a stable dose (>80% labelled dose/kg) for at least 6 months. At least 2 historical serum creatinine evaluations since starting agalsidase alfa treatment and not more than 2 years should be available.

Exclusion Criteria were history of anaphylaxis or Type 1 hypersensitivity reaction to agalsidase alfa; history of renal dialysis or transplantation; history of acute kidney injury (AKI) in the 12 months prior to screening; angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) therapy initiated or dose changed in the 4 weeks prior to screening; urine protein to creatinine ratio (UPCR) >0.5 g/g and not treated with an ACEi or ARB; cardiovascular event (myocardial infarction, unstable angina) in the 6-month period before screening; congestive heart failure New York Heart Association (NYHA) Class IV and cerebrovascular event (stroke, transient ischemic attack) in the 6-month period before screening.

The aim of this study was to evaluate the safety (primary objective) and efficacy (secondary objective) of pegunigalsidase alfa in patients with Fabry disease currently treated with agalsidase alfa. Secondary efficacy assessment included: mean annualized change in estimated glomerular filtration rate (eGFR), plasma and urine Lyso-Gb3 and plasma Gb3 concentrations, left ventricular mass index (LVMI), urine protein to creatinine ratio (UPCR), frequency of pain medication use, exercise tolerance (stress test), short form Brief Pain Inventory (BPI), Mainz Severity Score Index (MSSI), Fabry Clinical Events, and quality of life (EQ-5D-5L).

A total of 27 patients were screened, of whom 22 patients (15 males and 7 females) were enrolled and treated.

Fifteen patients (68.2%) were male, and 7 patients (31.8%) were female. Fourteen of the 15 male patients and none of the female patients showed a classic phenotype. Of note, it had been planned to include a maximum of 25% of female patients in the study, although no formal sample size calculation was performed for this study.

The mean (SE) annualized eGFR slope in the EP was -5.90 mL/min/1.73 m²/year (1.34) pre-switch and -1.19 mL/min/1.73 m²/year (1.77) post-switch. The mean (SE) change in annualized eGFR slope from pre- to post-switch was 4.70 mL/min/1.73 m²/year (2.26; p=0.051, paired t-test). Annualized eGFR slopes were similar and the changes in annualized eGFR slope from baseline to Month 12 were consistent in male and female patients.

The mean (SE) cardiac LVMI at baseline was above normal range in male patients (97.6 [8.9]) and within normal range in female patients (66.9 [5.8]). At Month 12, the mean LVMI in male patients remained fairly stable (98.3 (7.8]) while in female patients the mean LVMi increased by 7.1 g/m² to 74.1 (5.0) but remained within normal ranges.

The overall impression of the stress test (exercise tolerance) was normal in 13 patients (65.0%) and not normal in 7 patients (35.0%) at baseline and normal in 10 patients (52.6% of patients with data) and not normal in 8 patients (42.1% of patients with data) at Month 12; for 2 patients (1 with normal and 1 with non-normal results at baseline), no stress test results at Month 12 were available. Male patients were more likely to have normal stress test results at baseline (10 male patients, 76.9% versus 3 female patients, 42.9%). Only in male patients did changes occur from abnormal to normal (2 patients) or from normal to abnormal (4 patient).

A total of 14 patients (70.0%; 9 male patients and 5 female patients) used pain medication at baseline. Two patients (10.0%) taking no pain medication at baseline took 1 pain medication at the last visit and 1 patient taking a single pain medication at baseline could stop the intake by Month 12 Patients most often used 1 (6 patients, 30.0%) or 2 (5 patients, 25.0%) pain medications during the study. At a maximum 5 pain medications were taken during the study (1 patient, 5.0%).

The short form BPI results showed no major changes from baseline to Month 12 for the different categories, where zero (0) is no pain or interference. At baseline 11 patients (55.0%) had a pain on average score of zero or 1, (no or very mild pain). BPI results showed a mean (SE) change in the pain severity mean severity score from a baseline value of 1.43 (0.37) to Month 12 of 0.23 (0.21). The baseline value of the mean pain interference mean interference score was 1.53 (0.45) and change to Month 12 was and of -0.10 (0.29).

Cardiac (new complete right bundle branch block) and cerebrovascular (transient ischemic attack) Fabry Clinical Events were each reported in 1 patient (5.0%) in the EP. Both events occurred in the same male patient with a previous medical history of transient ischaemic attacks

The EQ-5D-5L descriptive results showed no major changes from baseline to Month 12 in the different dimensions.

PB-102-F50

Study PB-102-F50 was an open label, switch study to assess the safety, efficacy and PK of pegunigalsidase alfa 2.0 mg/kg administered by IV infusion E4W for 12 months in adult patients with Fabry disease who were previously treated with ERT. Enrolled patients had to have been treated prior to the study with agalsidase beta (Fabrazyme) or agalsidase alfa (Replagal) for at least 3 years, and to have been on a stable dose (> 80% labelled dose/kg) for at least 6 months.

Eligible patients included male and female patients aged ≥ 18 and ≤ 60 years with a documented diagnosis of Fabry disease who had been treated with agalsidase alfa or agalsidase beta for at least 3 years prior to inclusion (and for at least 6 months prior to inclusion at a stable dose > 80% labelled dose/kg).

All included patients were required to have an estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m^2 (according to the Chronic Kidney Disease – Epidemiology Collaboration [CKD-EPI] equation); patients with a linear negative eGFR slope of ≥ 2 mL/min/1.73 m^2/year were excluded.

The objective was to evaluate the safety, efficacy and pharmacokinetics (PK) of pegunigalsidase alfa (PRX-102) at a dosing regimen of 2.0 mg/kg E4W in patients with Fabry disease currently treated with currently commercially available enzyme replacement therapy (agalsidase alfa or agalsidase beta)._Efficacy Variables included:_eGFR CKD-EPI; eGFR slope; Plasma globotriaosylsphingosine (Lyso-Gb3); Plasma globotriaosylceramide (Gb3); Left ventricular mass index (g/m^2) by echocardiogram; Usage of pain medication; Stress test; Short Form Brief Pain Inventory (BPI); Mainz Severity Score Index (MSSI); Quality of life (QoL; EuroQol 5 Dimensions 5 Levels Questionnaire [EQ-5D-5L]); Fabry Clinical Events (FCEs). Urine Lyso-Gb3; Urine protein to creatinine ratio (UPCR) spot urine test.

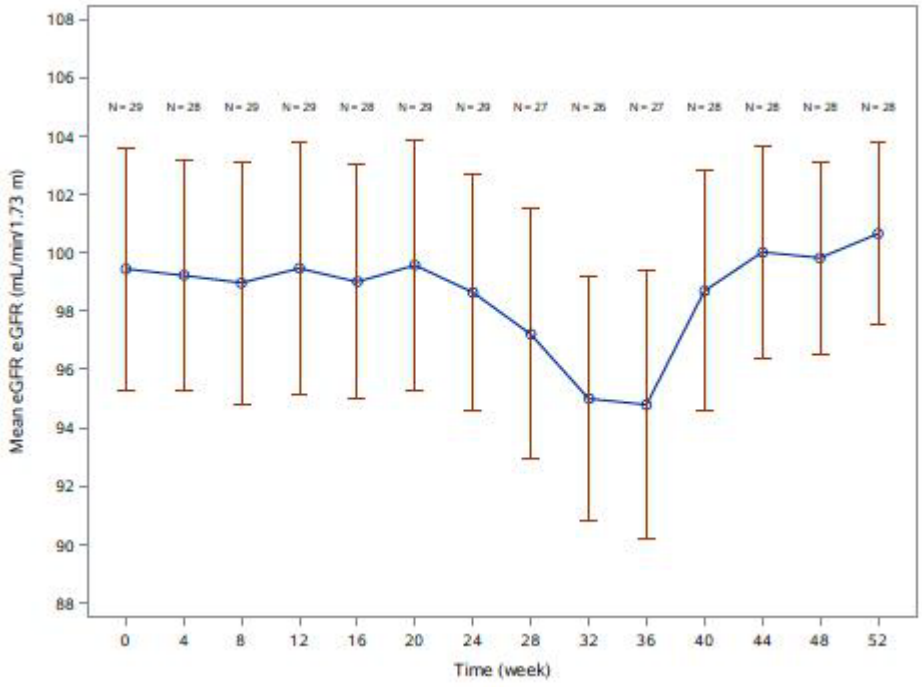
A total of 52 patients were screened, of whom 30 patients (24 males and 6 females) were enrolled and treated. Overall, 29 patients (23 males and 6 females) completed the study; 1 male patient discontinued as he withdrew his consent after receiving the first infusion of pegunigalsidase alfa 2.0 mg/kg.

The pegunigalsidase alfa dosing regimen was changed to 1.0 mg/kg E2W for 1 patient.

In all 30 enrolled and treated patients, the mean (SD) age was 40.5 (11.3) years, median 40.5 years, ranging from 19 to 58 years of age. Twenty-four (80.0%) patients were male and 6 (20.0%) patients were female, in line with the plan to include ~20% female patients in the study. Mean (SD) eGFR at baseline was 99.89 (22.08) mL/min/1.73 m² overall and was slightly higher in male (101.19 [23.38] mL/min/1.73 m2) than female (94.69 [16.55] mL/min/1.73 m²) patients. Mean (SD) annualized eGFR slope pre-switch was -1.84 (3.66) mL/min/1.73 m²/year overall. Patients with a linear slope more negative than -2 mL/min/1.73 m²/year, calculated using historical values and screening values, were excluded from the study at screening.

The efficacy population included 29 patients (23 males and 6 females). One patient who was excluded from the efficacy population discontinued the study (withdrew consent) after the first infusion, but prior to any post-baseline efficacy evaluation. Mean absolute eGFR values over time generally remained relatively stable during the study, with values of 99.44 (n=29) and 100.65 mL/min/1.73 m² (n=28) at baseline and Week 52, respectively. See Figure 18.

Figure 18 Mean (SE) eGFR Over Time – Efficacy Population



eGFR = Estimated glomerular filtration rate; SE = standard error.
Source: Figure 15.2.1.2

Annualized eGFR slopes were calculated at screening (based on historical and screening eGFR values), pre-switch (based on historical, screening and baseline [V1] eGFR values) and post-switch (based on baseline [V1] and all post-switch eGFR values). Values for mean annualized eGFR slopes at screening, pre- and post-

switch in the Efficacy population are presented overall, by gender and by ADA status, by Fabry disease classification, by previous ERT, by eGFR, by use of ACEi/ARB and by hyperfiltration status. See

Table 49.

Table 49 eGFR Slope Pre- and Post-switch Overall and by Subgroup – Efficacy Population

Population/Subgroup		Pre-switch	Post-switch
Efficacy Population			
	n	29	
	Mean (SE)	-1.79 (0.69)	-2.92 (1.05)
	Median (min ; max)	-1.06 (-13.6 ; 3.6)	-1.86 (-14.1 ; 8.2)
Gender			
Male			
	n	23	
	Mean (SE)	-1.15 (0.67)	-3.02 (1.22)
	Median (min ; max)	-0.64 (-10.5 ; 3.6)	-1.52 (-14.1 ; 8.2)
Female			
	n	6	
	Mean (SE)	-4.24 (1.93)	-2.53 (2.14)
	Median (min ; max)	-3.12 (-13.6 ; -0.5)	-4.31 (-7.7 ; 5.0)
95% CI for difference in post-switch means		(-5.9; 4.9)	
ADA status			
ADA negative			
	n	20	
	Mean (SE)	-2.31 (0.94)	-1.45 (1.11)
	Median (min ; max)	-1.67 (-13.6 ; 3.6)	-1.22 (-8.5 ; 8.2)
ADA positive			
	n	9	
	Mean (SE)	-0.63 (0.71)	-6.19 (1.99)
	Median (min ; max)	-0.64 (-4.3 ; 3.3)	-8.44 (-14.1 ; 2.7)
95% CI for difference in post-switch means		(0.4; 9.1)	
Fabry disease classification[1]			
Classic			
	n	15	
	Mean (SE)	-0.55 (0.89)	-2.55 (1.54)
	Median (min ; max)	0.08 (-10.5 ; 3.6)	-1.24 (-12.0 ; 8.2)
Non-classic			
	n	12	
	Mean (SE)	-3.24 (1.13)	-4.10 (1.59)
	Median (min ; max)	-2.87 (-13.6 ; 2.3)	-4.31 (-14.1 ; 5.0)
95% CI for difference in post-switch means		(-3.1; 6.1)	
Previous ERT			
Agalsidase alfa			
	n	7	
	Mean (SE)	-3.28 (2.02)	-0.97 (0.89)
	Median (min ; max)	-2.63 (-13.6 ; 2.6)	-1.52 (-3.1 ; 2.8)
Agalsidase beta			
	n	22	
	Mean (SE)	-1.31 (0.65)	-3.54 (1.33)
	Median (min ; max)	-1.03 (-10.5 ; 3.6)	-3.69 (-14.1 ; 8.2)
95% CI for difference in post-switch means		(-2.4; 7.6)	
Baseline eGFR			
≤ 60 mL/min/1.73 m²			
	n	2	
	Mean (SE)	-4.65 (0.35)	-0.24 (8.44)
	Median (min ; max)	-4.65 (-5.0 ; -4.3)	-0.24 (-8.7 ; 8.2)
> 60 mL/min/1.73 m²			
	n	27	
	Mean (SE)	-1.57 (0.72)	-3.12 (1.02)
	Median (min ; max)	-1.00 (-13.6 ; 3.6)	-1.86 (-14.1 ; 5.0)
95% CI for difference in post-switch means		(-5.7; 11.4)	
Use of ACEi/ARB			
Yes			
	n	10	
	Mean (SE)	-2.85 (1.25)	-2.38 (2.01)
	Median (min ; max)	-2.93 (-10.5 ; 3.3)	-1.38 (-11.3 ; 8.2)
No			
	n	19	
	Mean (SE)	-1.23 (0.82)	-3.20 (1.23)
	Median (min ; max)	-1.00 (-13.6 ; 3.6)	-2.65 (-14.1 ; 5.0)
95% CI for difference in post-switch means		(-3.8; 5.4)	
Hyperfiltration status			
Yes			
	n	5	
	Mean (SE)	-1.25 (0.52)	-5.41 (3.21)
	Median (min ; max)	-1.06 (-2.7 ; 0.1)	-1.86 (-14.1 ; 2.2)
No			
	n	24	
	Mean (SE)	-1.90 (0.83)	-2.40 (1.08)
	Median (min ; max)	-1.20 (-13.6 ; 3.6)	-2.08 (-11.3 ; 8.2)
95% CI for difference in post-switch means		(-8.7; 2.7)	

ACEi = Angiotensin-converting enzyme inhibitor; ADA = anti-drug antibody; ARB = angiotensin receptor blocker; CI = confidence interval; CKD-EPI = Chronic Kidney Disease – Epidemiology Collaboration; eGFR = estimated glomerular filtration rate; ERT = enzyme replacement therapy; max = maximum; min = minimum; SE = standard error.
1 Subgroup analyses by Fabry disease classification included a maximum of 28 patients; classification of the disease as "classic" or "non-classic" was not possible for the remaining 2 patients in the Safety population. eGFR calculated using the CKD-EPI equation; eGFR slope units are mL/min/1.73 m^2/year.
Source: Table 14.2.1.3.1, Table 14.2.1.3.2 and Table 14.2.1.3.3 and Table 14.2.1.3.5

Data for quantitative echocardiography evaluations, e.g., LVMI, had substantial variations. In addition, in this study the echocardiograms were not performed in a standardized manner and/or read centrally.

Other evaluated endpoints (Pain medication, Short Form Brief Pain Inventory, Mainz Severity Score Index, Quality of Life) were overall stable during the study. Only 1 patient (non-classic Fabry disease); previous ERT of agalsidase beta who was taking 1 pain medication at baseline was taking ≥ 2 medications by the last visit. Data at baseline, week 52 are presented further below with the interim extension study data (up to week 108).

Study PB102-F51

Study PB102-F51 is an ongoing open label, extension study that evaluates the safety and efficacy parameters of pegunigalsidase alfa 2.0 mg/kg E4W in adult Fabry patients who successfully completed Study PB-102-F50. Patients were enrolled to receive 2.0 mg/kg E4W as an intravenous (IV) infusion for up to 36 months (per protocol Version 3.0) or until marketing approval for pegunigalsidase alfa is obtained and the treatment is available in the patients' country or until study is terminated. In the case of clear clinical deterioration, the treatment regimen may be changed to 1.0 mg/kg every 2 weeks (E2W) at the Investigator's discretion and after discussion with the Medical Monitor. Patients who completed study PB-102-F50 were enrolled to this study.

All 29 enrolled patients are ongoing in Study PB-102-F51 at the time of this interim analysis cut-off date of 08 August 2021. Twenty-seven patients are receiving PRX-102 at 2 mg/kg E4W, while the PRX-102 dosing regimen has been changed to 1.0 mg/kg E2W for 2 patients: one patient was switched at Week 40 (V11) during Study PB-102-F50 and continued with the 1.0 mg/kg E2W regimen during this extension study; and another patient was switched at Week 84 (V22) (during Study PB-102-F51).

The safety population consisted of all patients who received any dose (partial or complete) of pegunigalsidase alfa as part of this PB-102-F51 study. All safety analyses were based on this population.

The efficacy population consisted of all patients who received any dose of pegunigalsidase alfa as part of this study. Only efficacy data collected while on the 2.0 mg/kg E4W regimen were included in the efficacy tables. For the patients who switched to the 1.0 mg/kg E2W dosing regimen, efficacy data collected after the switch were listed only.

All 29 patients included in the study were white of which 1 patient was Hispanic/Latino. A total of 23 (79.3%) patients were male and 6 (20.7%) patients were female. The mean (SD) age was 40.9 (11.3) years, ranging from 19 to 58 years. The most common Fabry disease symptoms/presentations were acroparesthesias, heat intolerance, angiokeratomas and hypohydrosis. The efficacy population included 29 patients with available efficacy data (23 males and 6 females).

A total of 15 (51.7%) patients were treated with ACEi and/or ARBs at any timepoint during the study. Of these, 10 patients were already being treated with ACEi/ARB at study entry and 5 patients started treatment during the study. Of the 10 patients already being treated at study entry, 8 patients continued treatment at a stable dose throughout the study.

The baseline characteristics of the study population indicated that the population was likely to be more variable than expected. This variability is possibly reflected in the heterogenicity of the observed eGFR results and the

less-than-optimal agreement between eGFR slope and eGFR values observed in some cases,and could be also impacted by some methodological limitation for the eGFR pre-switch slope calculation (e.g., creatinine assessments used coming from different laboratories and at varied intervals).

The mean absolute eGFR value at baseline (Study PB-102-F50) in the efficacy population was 99.44 mL/min/1.73 m2 and was slightly higher in male patients than female patients (100.68 and 94.69 mL/min/1.73 m2, respectively).

Estimated Glomerular Filtration Rate

Mean (SE) eGFR values and changes from baseline are summarized for the overall Efficacy population in Table 50. The eGFR changes from baseline remained relatively stable to Week 52 and decreased at Week 108 and subsequent timepoints, with mean change from baseline of -1.27 at Week 52 and -5.10 mL/min/1.73 m^2 at Week 108. See Table 50.

Table 50 Mean (SE) eGFR Values and Changes from Baseline- Overall Efficacy Population

		Overall N=29	
		eGFR[1] mL/min/1.73 m^2	
Week (Visit)	n	Mean (SE)	Mean (SE) Change from Baseline
Baseline (V1)	29	99.44 (4.15)	N/A
Week 52 (V14)	28	100.65 (3.14)	-1.27 (1.39)
Week 108 (V28)	26	95.13 (3.38)	-5.10 (1.96)
Week 132 (V34)	11	91.26 (5.25)	-6.77 (3.43)
Week 160 (V41)	17	97.46 (3.95)	-10.45 (3.05)
Week 184 (V47)	10	102.21 (4.32)	-6.57 (3.60)

eGFR calculated using the CKD-EPI equation; units are mL/min/1.73 m2.
Note: Week 80 (V21) data are not included in this table due to the low number of patients (N=4) with measurements at this timepoint.
Source: Table 14.2.1.1.1

Estimated Glomerular Filtration Rate Slope

Data are presented in Table 51.

Table 51 eGFR Slope at Baseline and During Treatment- Overall Efficacy Population

Population/Subgroup		Baseline	During Treatment
Efficacy population			
	n	29	
	Mean (SE)	-1.79 (0.69)	-2.77 (0.54)
	Median (min ; max)	-1.06 (-13.6 ; 3.6)	-2.47 (-8.7 ; 1.4)
Gender			
Male	n	23	
	Mean (SE)	-1.15 (0.67)	-3.03 (0.61)

	Median (min ; max)	-0.64 (-10.5 ; 3.6)	-2.82 (-8.7 ; 1.4)
Female	n	6	
	Mean (SE)	-4.24 (1.93)	-1.74 (1.21)
	Median (min ; max)	-3.12 (-13.6 ; -0.5)	-1.45 (-6.5 ;1.4)
95% CI for difference in post-switch means		(-4.1; 1.5)	

ACEi = angiotensin-converting enzyme inhibitor; ADA = anti-drug antibody; ARB = angiotensin receptor blocker; CI = confidence interval; CKD-EPI = Chronic Kidney Disease _ Epidemiology Collaboration; eGFR = estimated glomerular filtration rate; ERT = enzyme replacement therapy; max = maximum; min = minimum; SE = standard error.
[1] Subgroup analyses by Fabry disease classification included a maximum of 27 patients.
eGFR calculated using the CKD-EPI equation; eGFR slope units are mL/min/1.73 m^2/year.
Source: Table 14.2.1.3.1, Table 14.2.1.3.2, Table 14.2.1.3.3 and Table 14.2.1.3.4

Subgroup analysis on eGFR and its slope were performed by gender, kidney hyperfiltration Status ADA status and Fabry disease classification. No clear pattern regarding an improvement for some subgroups can be drawn. Due to the small number of patients and the heterogenicity of the subgroups, such analyses and observations should be interpreted with caution. Presentation of the data on the subgroup analysis by gender for the eGFR slope is presented above.

Plasma Lyso-Gb3

Mean (SE) plasma Lyso-Gb3 concentrations and changes from baseline are summarized for the Efficacy population in Table 52. The mean plasma Lyso-Gb3 concentrations remained relatively stable from baseline (Study PB-102-F50), with a slight increase from baseline to Week 24 (V14) and stability through to Week 108 (V28). See Table 52.

Table 52 Mean (SE) Plasma Gb3 Concentrations and Changes from Baseline Efficacy Population by gender

	Male N=23			Female N=6		
		Plasma Lyso-Gb3 (nM)			Plasma Lyso-Gb3 (nM)	
Week (Visit)	n	Mean (SE)	Mean (SE) Change from Baseline	n	Mean (SE)	Mean (SE) Change from Baseline
Baseline (V1)	23	23.27 (3.82)	N/A	6	4.35 (1.00)	N/A
Week 52 (V14)	22	27.05 (4.00)	3.79 (1.14)	6	4.52 (1.10)	0.17 (0.34)
Week 108 (V28)	20	27.30 (4.11)	4.03 (1.59)	5	5.74 (1.07)	0.66 (0.78)
Week 132 (V34)	9	29.48 (8.75)	4.82 (2.42)	3	3.53 (1.85)	-0.67 (0.20)
Week 160 (V41)	14	31.64 (6.27)	6.66 (2.49)	3	5.13 (1.17)	0.63 (0.49)
Week 184 (V47)	7	27.54 (4.58)	2.34 (3.86)	3	5.87 (1.70)	1.37 (0.98)

Lyso-Gb3 = globotriaosylsphingosine; n = number of patients; N = number of patients overall; N/A = not applicable; SE = standard error; V = visit.
Note: Week 80 (V21) data are not included in this table due to the low number of patients (N=4) with measurements at this timepoint.
Source: Table 14.2.2.1.1

Plasma Gb3 concentration

Mean (SE) plasma Gb3 concentrations and changes from baseline are summarized for the overall Efficacy population in Table 53. The mean plasma Gb3 concentrations remained relatively stable from baseline (Study PB-102-F50) to Week 108. See Table 53.

Table 53 Mean (SE) Plasma Gb3 Concentrations and Changes from Baseline by Gender Efficacy Population

	Male N=23			Female N=6		
		Plasma Gb3 (nM)			Plasma Gb3 (nM)	
Week (Visit)	n	Mean (SE)	Mean (SE) Change from Baseline	n	Mean (SE)	Mean (SE) Change from Baseline
Baseline (V1)	23	4464.4 (309.28)	N/A	6	4499.5 (480.18)	N/A
Week 52 (V14)	21	5083.2 (390.40)	488.95 (226.77)	6	4292.2 (708.20)	-207.3 (271.44)
Week 108 (V28)	19	4262.1 (344.65)	-220.2 (233.99)	5	4308.4 (786.01)	-199.2 (318.72)
Week 132 (V34)	9	5041.1 (803.34)	542.67 (345.47)	3	4303.3 (1025.9)	-825.7 (232.97)
Week 160 (V41)	14	4653.2 (509.70)	175.71 (324.78)	3	3448.3 (395.06)	-421.7 (233.94)
Week 184 (V47)	7	5086.6 (457.63)	291.86 (416.58)	3	3977.3 (811.68)	107.33 (765.39)

Gb3 = globotriaosylceramide; n = number of patients; N = number of patients overall; N/A = not applicable; SE = standard error; V = visit.
Note: Week 80 (V21) data are not included in this table due to the low number of patients (N=4) with measurements at this timepoint.

As in the original study PB-102-F50, data for quantitative echocardiography evaluations, e.g., LVMI, have substantial variations. In addition, in this study the echocardiograms were not performed in a standardized manner and/or read centrally.

Of the 15 patients taking no pain medication at baseline, 4 (26.7%) patients and 1 (6.7%) patient were taking 1 and 2 pain medications, respectively, at Week 52 and 3 (20.0%) patients and 1 (6.7%) patient took 1 and 2 pain medications, respectively, at Week 108; Of the 6 patients taking 1 pain medication at baseline, 4 (66.7%) patients took 2 pain medications at Week 52 and 2 (33.3%) patients took 2 pain medications at Week 108.

Other evaluated endpoints (Short Form Brief Pain Inventory, Mainz Severity Score Index, Quality of Life) were overall stable during the study. Mean worst pain scores change from baseline were 0.4 at Week 52 and 0.5 at Week 108; mean least pain scores change from baseline were 0.3 at Week 52 and 0.1 at Week 108; mean average pain scores were 0.1 at Week 52 and -0.0 at Week 108; and mean pain right now scores change from baseline were of -0.0 at Week 52 and 0.2 at Week 108. Mean total general MSSI scores were 5.5, 5.2 and 4.9 at baseline, Week 52 and Week 108, respectively, with mean change from baseline of -0.2 at Week 52 and -0.4 at Week 108. The mean (SE) EQ-5D-5L overall health scores were 78.3, 81.9 and 80.2 at baseline, Week 52 and Week 108, respectively, with a mean change from baseline of 2.9 at Week 52 and 0.5 at Week 108.

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

Over the course of the procedure, the applicant submitted the following studies/data:

- A Phase 1/2 study in adult ERT-naïve or off-ERT for 6 months Fabry patients; PB-102-F01/F02 followed by an extension study (PB-102-F03);
- Interim and Final results of a Phase 3 main study in previously ERT-treated adult Fabry patients after switch from Fabrazyme (PB-102-F20);
- A Phase 3 supportive study in previously ERT-treated adult Fabry patients after switch from Replagal (PB-102-F30);
- A Phase 3 supportive study investigating the alternative dose of 2.0 mg/kg E4W in ERT pre-treated patients (PB-102-F50);
- Interim report of an open label extension study to evaluate long term safety and efficacy of Pegunigalsidase Alfa (PRX-102) 2.0 mg/kg E4W (PB-102-F51).

The proposed indication is: “Elfabrio is indicated for long-term enzyme replacement therapy in adult patients with a confirmed diagnosis of Fabry disease (deficiency of alpha-galactosidase).”

STUDY PB102-20 (Interim and Final analyses)

STUDY PB-102-20 was a randomized, double-blind, multicentre, active control study examining efficacy and safety of pegunigalsidase alfa in adult Fabry disease patients with impaired renal function and previously treated with agalsidase beta (Fabrazyme).

During the EMA scientific advice meeting in December 2015 [EMA SA, 2015] it was acknowledged that a 12-month interim analysis could constitute a pivotal evidence for efficacy, if all aspects of the study were appropriately designed. The applicant consequently used the 12-month interim analysis as primary objective of the EU submission.

In the original planned study, the primary final analysis was a test for superiority over Fabrazyme at 24 months to support the requirement of another regulatory authority and was considered as a secondary objective for the EU dossier. During the procedure, the final clinical study report of PB-102-F20 was submitted. The CHMP noted a change in the objective of the final (24-month) analysis from superiority to non-inferiority and a change in the primary analysis model (from a random intercept random slope “RIRS” longitudinal mixed model comparing adjusted means of eGFR slopes to a quantile regression comparing medians of eGFR slopes) from the original SAP.

Currently 3 products are registered for adult patients with Fabry disease: 2 ERTs agalsidase beta (Fabrazyme) and agalsidase alfa (Replagal) and a pharmacological chaperon (migalastat [Galafold]). The use of an active comparator (agalsidase beta) is considered acceptable by the CHMP.

The population included in this study is considered adequately selected:

- the population covers female and male patients with classic and non-classic Fabry disease, with renal function deterioration (Linear slope of eGFR more negative than -2 mL/min/1.73 m²);
- no more than 50% of patients were female which is appropriate since the disease expresses differently in males and females, and the symptoms are usually milder in females.

Patients were randomized in a 2:1 ratio to either receive pegunigalsidase alfa 1 mg/kg EOW or to continue agalsidase beta at the same dosage and interval as previously and patients were previously treated with agalsidase beta with a high compliance.

The primary endpoint was the annualised estimated glomerular filtration rate eGFR (defined using the CKD-EPI, Chronic Kidney Disease-Epidemiology Collaboration formula) over time or so called annualised eGFR slope. The baseline slope was determined based on at least 3 historical serum creatinine values over approximately 1 year. The selection of the primary endpoint was based on experience from pre-clinical studies and the clinical phase I/II study, this endpoint was also chosen in the phase III study for the latest approved EU product in this indication (migalastat) and is considered acceptable by the CHMP.

Secondary endpoints included plasma and urine Lyso-Gb3 and plasma Gb3 concentrations, left ventricular mass index (LVMI), urine protein to creatinine ratio (UPCR), frequency of pain medication use, exercise tolerance, short form Brief Pain Inventory (BPI), Mainz Severity Score Index (MSSI), Fabry Clinical Events, quality of life (EQ-5D-5L) and biomarkers and are considered relevant to support the efficacy of pegunigalsidase alfa.

The sample size calculation was based on superiority and non-inferiority hypotheses and these elements of the study design raised CHMP major concerns. Indeed, in the non-inferiority objective, any treatment effect of which magnitude does not exceed 3 mL/min/1.73 m²/year is considered as not clinically relevant, according to the non-inferiority margin in the trial. On the other hand, both non-inferiority and superiority objectives are targeting the same 1.1 mL/min/1.73 m²/year difference in favour of pegunigalsidase alfa considered as a clinically relevant treatment effect in the superiority settings. However, when considering the clinical assumptions set in the protocol (pegunigalsidase alfa =-1.9, agalsidase=-3.0, Δ=1.1, sd =1.5 and 66 subjects), the magnitude of the targeted treatment effect seems to be within a range of non-clinically relevant values, according to the 95% confidence limits of the point estimate of the difference Δ ([0.32; 1.88]) and the non-inferiority margin. Given the treatment effect expected in favour of pegunigalsidase alfa for superiority as well as non-inferiority objectives (1.1 mL/min/1.73 m²/year), the trial would have had more than 90% power to achieve the non-inferiority with a margin as small as -0.5 or -0.25 mL/min/1.73 m²/year.

The CHMP concluded that the design of the main Study PB-102-F20 was considered inadequate to test the non-inferiority (NI) of Elfabrio vs. its comparator. The prespecified NI margin was based on the absolute effect of the comparator in one experience, rather than on retaining a modicum of its effect in comparison to no treatment. First, this NI margin (-3 mL/min/1.73 m²/year) implies that a loss of efficacy of PRX-102 over the comparator as high as three times the expected gain in efficacy (+1 mL/min/1.73 m²/year) has no clinical relevance. Second, the key clinical data of the approval of algasidase beta (Fabrazyme) was the superiority over untreated patients with a mean eGFR slope difference of 1.7 mL/min/1.73 m2/year within a 95% CI of [0.5, 3.0]. This result shows that the maximum gain in efficacy can reach 3 mL/min/1.73 m2/year as compared to untreated patients and therefore makes difficult to understand how a maximum gain in efficacy can be considered as a negligible loss of efficacy in a non-inferiority trial. Thus, available evidence on the effect of Fabrazyme over no treatment indicate that a robust NI margin would need to be so small, that this would be prohibitive with respect to sample size. Further, the different data cited on the effect of Fabrazyme from different sources, indicate that “constancy of assumptions” may not be mandated and thus, the presented main trial is an experiment that could only have established the efficacy of Elfabrio if superior efficacy had been shown, which was not the case.

Subsequently, additional available pharmacodynamics and efficacy analyses were presented by the applicant and the CHMP considered the presented results to conclude on the efficacy based on the totality of the evidence. See further details below.

PB-102-F01/ PB-102-F02 / PB-102-F03

Dose selection for the main Phase 3 was based on pharmacokinetics, pharmacodynamics/biomarker, efficacy, and safety data from study PB-102-F01/ PB-102-F02 /PB-102-F03.

This study was the only one to have included ERT-naive patients.

The primary objective was to evaluate the safety and tolerability of different dosages (0.2; 1.0 and 2.0 mg/kg QOW) of pegunigalsidase alfa in patients with Fabry disease. Secondary objectives included pK and exploratory efficacy endpoints.

Inclusion criteria were male and female Fabry disease patients who were required to either be ERT-naïve or to have not received ERT within the last 6 months.

Only descriptive statistics for continuous variables (mean and its standard error, standard deviation, median and range) were presented as efficacy results.

PB-102-F30

Additional evidence of efficacy in patients treated with agalsidase alfa came from Study PB-102-F30, BRIDGE, which was an open-label, switch study aimed to assess the safety and efficacy of pegunigalsidase alfa in adult Fabry disease patients treated with the other currently commercially available ERT agalsidase alfa (Replagal).

The objective of this study was to evaluate the safety (primary objective) and efficacy (secondary objective) of pegunigalsidase alfa. Efficacy objectives comprised mean annualized change in estimated glomerular filtration rate (eGFR) and also renal, cardiac, pain, quality of life and PD evaluations.

Inclusion criteria were male and female patient with Fabry disease. Patients had to have been receiving agalsidase alfa for at least 2 years and a stable dose (>80% of the labelled dose/kg) for at least 6 months.

Only descriptive statistics for continuous variables (mean and its standard error, standard deviation, median and range) were presented for efficacy. A subgroup analysis of patients with classic Fabry, males and females was conducted.

PB-102-F50

Evidence regarding an alternative dose regimen (2.0 mg/kg E4W) came from Study PB-102-F50 which was an open label, switch over study aimed to assess the safety, efficacy and PK of pegunigalsidase 2.0 mg/kg administered by IV infusion E4W for 12 months in adult patients with Fabry disease previously treated with ERT.

The primary objective of this study was to evaluate the safety of an alternative dosing regimen as a maintenance therapy in patients currently treated with ERT. Efficacy objectives comprised mean annualized change in estimated glomerular filtration rate (eGFR) and also renal, cardiac, pain, quality of life and PD evaluations.

The study aimed to enrol a population with a milder Fabry disease and therefore, unlike the main study, patients with a linear eGFR of ≤ -2 mL/min/1.73 m²/year were excluded.

This study was descriptive in nature and no primary efficacy variable was defined. A subgroup analysis of patients with classic Fabry disease was conducted in males and females.

STUDY PB102-51

The study aimed to enrol patients who had completed treatment with pegunigalsidase alfa in Study PB-102-F50 for an additional 36-month treatment period.

The objective of this study was to evaluate the long term safety and efficacy of pegunigalsidase 2.0 mg/kg administered by IV infusion E4W. Efficacy objectives comprised mean annualized change in estimated glomerular filtration rate (eGFR) and also renal, cardiac, pain, quality of life and PD evaluations

Only descriptive statistics for continuous variables (mean and its standard error, standard deviation, median and range) were presented for efficacy.

Efficacy data and additional analyses

STUDY PB102-20

Interim analysis (Month 12)

A total of 127 subjects were assessed for inclusion and 78 were enrolled and randomized. At 12 months, The PP population comprised 74 patients (49 in the pegunigalsidase alfa arm and 25 in the agalsidase beta arm).

At baseline, males outnumbered females in both arms, but with a disparity between arms. The number of male patients was lower in the pegunigalsidase alfa 29 (55.8%) arm than in the in the agalsidase beta arm 18 (72.0%). Since the disease expresses differently in males and females, and as the symptoms are usually milder in females this imbalance may result in a bias.

Characteristic data at baseline suggest that the proportion of patients with a more severe form of the disease was greater in the agalsidase arm:

- the overall mean (SE) eGFR was 73.33 (2.26) mL/min/1.73 m² with comparable means. However, the proportion of patients with a more pronounced decline in kidney function, as measured by an annualised eGFR slope below -5 was higher in the agalsidase alfa arm than in the pegunigalsidase alfa arm (80.0% and 63.5% respectively);
- more patients were taking ACEi or ARB medications in the agalsidase beta arm than the pegunigalsidase alfa arm (64.0% and 51.9% respectively).

Around one third of patients had ADAs directed against the product to which they have been exposed: 34.6% of the patients were positive for pegunigalsidase alfa antibodies and 32.0% of the patients were positive for agalsidase beta antibodies. All patients had been on agalsidase beta prior to enrolment and as the protein structure of the two products are the same it is likely that the positive results for pegunigalsidase alfa antibodies were due to antibody cross-reactivity.

At Month 12, the estimated eGFR slopes were -2.507 for the pegunigalsidase alfa arm and -1.748 for the agalsidase beta arm in favour of agalsidase beta [difference -0.749 (-3.026; 1.507)]. For the per-protocol population, the LS mean annualised eGFR estimated slope (95%CI) was -2.403 (-3.714; -1.093) mL/min/1.73m², for the pegunigalsidase alfa compared to -1.755 (-3.556; -0.056) mL/min/1.73m² for the agalsidase beta group with a difference of -0.648 (-2.883 to 1.587) in favour of algalsidase beta.

The non-inferiority criterion was not met for the ITT set since the lower limit of the 95% confidence interval (CI) had to be greater than the pre-specified non-inferiority margin of -3.0. Indeed, the 95% CI for the

difference in slopes was -3.026 to 1.507. Since non-inferiority could not be demonstrated for the primary endpoint, all subsequently planned statistical tests for the secondary endpoints in the pre-specified hierarchy could not be carried out under adequate control of the experiment-wise type-1-error. Thus, from a methodological/statistical point of view, no firm conclusions can be drawn on the efficacy of pegunigalsidase alfa as compared to that of agalsidase beta and moreover the data do not allow to clearly quantify the effect of pegunigalsidase alfa.

The evolution of the annualised eGFR slope before and after randomisation in the study shared the same pattern in both arms: -8.08 (0.91) mL/min/1.73m² at baseline and -2.57 (95CI -3.835;-1.180) mL/min/1.73m² at month 12 in the pegunigalsidase alfa arm; -8.48 (0.95) mL/min/1.73m² at baseline and -1.74 (95CI -3.585;-0.089) mL/min/1.73m² at month 12 in the agalsidase beta arm. However, the intra-patient comparisons are fraught with uncertainty due to likely regression to the mean therefore the comparison of pre- baseline and post- baseline eGFR slopes may be biased. Indeed, the reliability of the data used to evaluate the pre-baseline slopes eGFR is debatable as historical data were obtained at screening through standard local laboratory procedures, whereas creatinine values within the study were obtained using an enzymatic assay performed at a central laboratory.

Separate presentation of results on biomarkers of Fabry disease for the different ADA-subgroups (Study F-20 only) seemed as if no effect of ADA development were on these efficacy outcomes. Difference in plasma Lyso Gb3 levels in male and female patients might have impact on the plasma Lyso Gb3 results in ADA-positive or induced ADA patients, if there are female patients in the ADA-positive/induced ADA subgroups and difference in Lyso-Gb3 induced by the ADA development might be masked by the intersexual differences. Thus no conclusions could be drawn to a possible advantage of pegunigalsidase alfa on the drug immunogenicity profile (see further discussion on safety).

Final analysis (Month 24)

At 24 months, the PP population comprised 72 patients (48 in the pegunigalsidase alfa arm and 24 in the agalsidase beta arm) and the ITT population comprised 77 patients (52 in the pegunigalsidase alfa arm and 25 in the agalsidase beta arm).

At month 24, an improvement in eGFR slope was observed in patients treated with pegunigalsidase alfa with a mean eGFR slope of -2.5 mL/min/1.73 m²/year at month 24 compared to -8.03 mL/min/1.73 m²/year before switch indicating less deterioration in renal function. The mean difference in the estimated slopes between pegunigalsidase alfa and agalsidase beta at month 24 was: -0.359 with a 95% CI [-2.444 ; 1.726] in the ITT population and -0.118 with a 95% CI [-2.450 ; 2.213] in the PP population.

PB-102-F01/ PB-102-F02

Up to 24 adult patients were planned to be enrolled into this study with a minimum of 4 males and 2 females per dose cohort. Overall 16 patients were included in the efficacy population 6 patients in the 0.2 and 1.0 mg/kg arms (4 males and 2 females each) but only 4 patients were included in 2.0 mg/kg group (3 females, 1 male) as the Applicant decided to stop enrolment in the 2.0 mg/kg treatment group. According to the Applicant, the decision to stop recruitment was based on PK data and not on safety issue. Considering the gender imbalance between the treatment arms, the 2.0 mg/kg arm cannot be compared to the other two arms. At Month 12, the percent reduction from baseline in Lyso-Gb3 was greater in the 1.0 mg/kg arm (mean -51.6 ng/mL; -59.9%) compared to the 0.2 mg/kg arm reduction (mean -59.7 ng/ml (-43.4%). Analysis of the renal Gb3 inclusions showed reduction in BLISS score from baseline in patients receiving pegunigalsidase alfa. No clear dose/effect relationship could be observed since the applicant only provided descriptive results.

PB-102-F30

The study showed a positive trend (stabilisation or improvement). The results are complicated to interpret due to the lack of a control arm and concerns about the reliability of the historical data used to calculate the annualized eGFR slope observed in the main study. Considering the underlying disease mechanism and the mechanism of action of pegunigalsidase alfa, this study could be considered sufficient for a switch from agalsidase alfa to pegunigalsidase alfa provided that the main phase data allow to conclude on the efficacy of pegunigalsidase alfa in patients with Fabry disease. Overall, 22 patients were included in this efficacy population (15 males and 7 females) versus Replagal. Fourteen of the 15 male patients and none of the female patients had classic Fabry disease. Renal function was assessed via eGRF and Annualized Estimated eGRF Slope (as in the main study versus Fabrazyme).

Overall the eGFR from baseline to Month 12 decreased by -2.56 (2.14) mL/min/1.73 m². The mean (SE) annualized eGFR slope was -5.90 mL/min/1.73 m²/year (1.34) pre-switch and -1.19 mL/min/1.73 m²/year (1.77) post-switch indicating improvement of 4.70 mL/min/1.73 m2/year (2.26; p=0.051, paired t-test).

Regarding the cardiac function evaluation (LVMI and stress test), a slight increase in LMVI was observed. Proportion of patients showing a normal stress test decreased from 65.0% at BL to 52.6% at Month 12. Similarly to the main study F-20, the increase of LVMI was numerically greater in female patients than in males, but remained within normal ranges from BL to study completion in female patients.

PB 102-F50

Data for an alternative dosing regimen (2.0 mg/kg/Q4W) come from an open-label phase 3, switch study evaluating safety (primary objective) and efficacy (secondary objective) of this new regimen in patients pretreated with ERTs. This alternative regimen was never tested in the dose finding studies and it is difficult to draw any conclusion taking into account the lack of a control arm and concerns about the reliability of the historical data used to calculate the annualized eGFR slope as acknowledged by the Applicant.

Overall, 29 patients (23 males and 6 females) completed this open label study aiming at assessing the 2.0 mg/kg IV every 4 weeks regimen (instead of 1.0 mg/kg IV every 2 weeks which was the dose regimen chosen for the main study).

Per protocol, patients with a negative eGFR slope of ≥ 2 mL/min/1.73 m²/year at screening (based on historical and screening values) were excluded. However, at baseline eGFR slopes (based on historical, screening and baseline values) negative eGFR slope of ≥ 2 were observed in some populations such as female patients, patients previously treated with agalsidase alfa, patients with non-classic Fabry disease and patients with an eGFR ≤ 60 mL/min/1.73 m². This data again raises the question of the accuracy of historical data.

The mean (SE) annualized eGFR slope values slightly decreased from -1.79 (0.69) mL/min/1.73 m²/year pre-switch to -2.92 (1.05)mL/min/1.73 m²/year post-switch showing a more negative eGFR slope post-swich. These results may have been biased by the possible unreliability of the historical data used to calculate the pre-switch slope.

In the efficacy population, the absolute eGFR values slightly decreased during the 52-week treatment period with pegunigalsidase alfa, with a mean (SE) change from baseline at 52 weeks of -1.27 (1.37) mL/min/1.73 m².

The cardiac function was generally stable over the study, with no major changes observed in qualitative echocardiography assessments. However these were not performed in a standardized manner and/or read

centrally. No major changes in pain perception was observed over the treatment period, no major changes were observed in the EQ-5D-5L descriptive results or overall health score.

Overall, the data from this study does not allow to draw a conclusion on the efficacy of this alternative dosing regimen for the following reasons:

- this was an open-label, uncontrolled study design;
- efficacy data were only descriptive;
- there were uncertainties about the accuracy of the historical data used to calculate the pre-switch slope, which makes the evolution of pre and post switch eGFR slopes comparison questionable.

STUDY PB102-F51

Further data for an alternative dosing regimen (2.0 mg/kg/Q4W) come from interim results of an open-label extension study evaluating safety, efficacy and PK of this new regimen in patients previously treated in study PB102-F50.

Overall, 29 patients (23 males and 6 females) were included all were treated for at least 108 weeks and 2 patients switched to the 1.0 mg/kg E2W dosing regimen.

For exploratory efficacy endpoints, the baseline values were defined as those collected at baseline in Study PB-102-F50. Thus, results at week 52 are those of the end of the study PB102-F50.

The mean (SE) annualized eGFR slope values stayed stable from -2.92 (1.05)mL/min/1.73 m²/year at baseline to -2.77 (0.54) mL/min/1.73 m²/year during treatment.

The eGFR decreased from baseline to Week 108 with mean (SE) change from baseline -5.10 (1.96) mL/min/1.73 m2 at Week 108.

The pain severity, the Mainz severity score index and the EQ-5D-5L remained generally stable from baseline to Week 108.

Regarding PD, comparison with Fabrazyme is not relevant for justifying the E4W posology. Furthermore, the provided population PK models is not endorsed and those data should not be used to characterise and predict the PK of pegunigalsidase alfa and are not accepted to be used to draw any conclusion on the PK, PK/PD and exposure-response behaviour of the compound and to recommend the Q4W posology.

Additional analyses

An integrated analysis of annualised eGFR slope including data across the development program of 1 mg/kg EOW, compared with originally naïve patients, and across all studies, with both the tested dose regimens has been generated. Only patients with at least 18 months of exposure were included. The integrated analysis covers treatment of up to 7.6 years with 1 mg/kg EOW. The mean eGFR slope was -2.80 mL/min/1.73 m²/year in the overall population as well as in the 1 mg/kg EOW dosing and -2.56 mL/min/1.73 m²/year in previously untreated patients.

In further additional analyses, most patients maintained normal or near normal Left ventricular mass index (LVMI) values throughout the studies. Mean changes were small and did not suggest deterioration of LVMI values over treatment. The severity of Fabry disease signs and symptoms according to the Mainz Severity Score Index (MSSI) and average pain severity remained stable in the switch studies.

2.6.7. Conclusions on the clinical efficacy

The CHMP concluded that no confirmatory evidence of efficacy as measured by the annualised Glomerular Filtration Rate (eGFR) can be retrieved from the main study given that the primary objective was not met. Despite this, pegunigalsidase alfa is a known active substance used as enzyme replacement therapy for Fabry disease with a well-understood mechanism of action. The pharmacodynamic effects of Elfabrio observed on renal tissue would not be expected without effective ERT, this is further supported by the observed clinical effect on the renal function at month 24. Taking into account the totality of the data, the CHMP concluded that efficacy of pegunigalsidase alfa has been shown in adult patients with Fabry disease (deficiency of alpha-galactosidase) in the proposed dosing regimen (1 mg/kg/EOW). Subsequent to the assessment of the data related to the (2 mg/kg/Q4W), the applicant withdrew this claim and subsequently no information in this dosing regimen should be included in the SmPC.

2.6.8. Clinical safety

The overview of the safety-relevant clinical studies is presented in Table 54.

Table 54 Overview of Safety-relevant Clinical Studies and Safety Endpoints

Study Number	Status	Number of Treated Patients	Dose (mg/kg)	Baseline Treatment	Safety Endpoints
PB-102-F01/ PB-102-F02	Completed	18	0.2, 1.0, 2.0 EOW	Naïve[a]	• TEAEs • Clinical laboratory (haematology, coagulation profile, biochemistry and urinalysis) safety tests • Anti-PRX-102 antibodies • ECG • Physical examination findings • Injection site reactions[b]
PB-102-F03	Completed	15	1.0 EOW (0.2, 2.0 during gradual dose adjustment)	Extension of F01/F02	• TEAEs (including IRR) • Clinical laboratory safety tests • Anti-PRX-102 antibodies • ECG • Physical examination findings • Injection site reactions[b] • Cerebrovascular disease assessment at M 24 and end of study (60 M).
PB-102-F20	Ongoing (blinded) Interim analysis covering 12-months included in MAA	77 (52 with pegunigalsidase alfa, 25 with Fabrazye)	1.0 EOW	Fabrazyme	• TEAEs (including IRR) • Clinical laboratory safety tests • Anti-PRX-102 antibodies • ECG • Physical examination findings • Injection site reactions[b] • Ability to taper off infusion premedication up to Month 2. • Requirement for use of premedication overall to manage infusion reactions
PB-102-F30	Completed	22	1.0 EOW	Replagal	Same as in PB-102-F20
PB-102-F60	Ongoing	69 (n=10 from F03, n=18 from F30, n=41 from F20[c])	1.0 EOW	OLE of F20, F30, and F03	Same as in PB-102-F20
PB-102-F50	Completed	30	2.0 E4W	Fabrazyme or Replagal	Same as in PB-102-F20
PB-102-F51	Ongoing	29	2.0 E4W	OLE of F50	Same as in PB-102-F20

ECG: Electrocardiogram; EOW: every other week; E4W: every 4 weeks; IRR: infusion-related reactions; OLE: open-label extension; M: month; MAA: Marketing Authorisation Application; TEAE: Treatment-emergent adverse event

a. Naïve: patients not previously exposed to an ERT or off-treatment for at least 6 months
b. Injection site reactions are provided with the Clinical Study Reports (CSRs) but not in this Safety Summary as they are often considered related to procedures rather than study treatment. See 2.7.4.3.5.1 for the definition of and results for the relevant safety endpoint Infusion Related Reactions (IRRs)
c. 26 patients coming from pegunigalsidase alfa arm and 15 from Fabrazyme arm of study F20

Reference: Individual CSRs or Study Protocols in 5.3.5, ISS Table 1.1.

The analysis sets (Cohorts) for the integrated, pooled analyses of safety data include only the subjects having received at least one complete or partial dose of pegunigalsidase alfa. Given the differences in dose/regimen and treatment historic of the populations enrolled and the different study designs in the clinical development program, 5 Cohorts were defined for the integrated analysis of safety. See

Table 55.

Table 55 Cohorts Defined for Integrated Safety Analyses

Cohort	Cohort Description	Studies Included in the Cohort: PB-102-
1	1 mg/kg PRX-102 EOW, all studies	F01, F02, F03*, F20 (PRX-102 arm), F30, F60
2	Switchers from other ERTs to 2 mg/kg PRX-102 E4W	F50, F51
3	Any dose and frequency of PRX-102	F01, F02, F03, F20 (PRX-102 arm), F30, F60, F50, F51
4	Switchers from other ERTs to 1 mg/kg PRX-102 EOW	F20 (PRX-102 arm), F30, F60
5	Naïve patients receiving 1 mg/kg PRX-102 EOW	F01, F02, F03

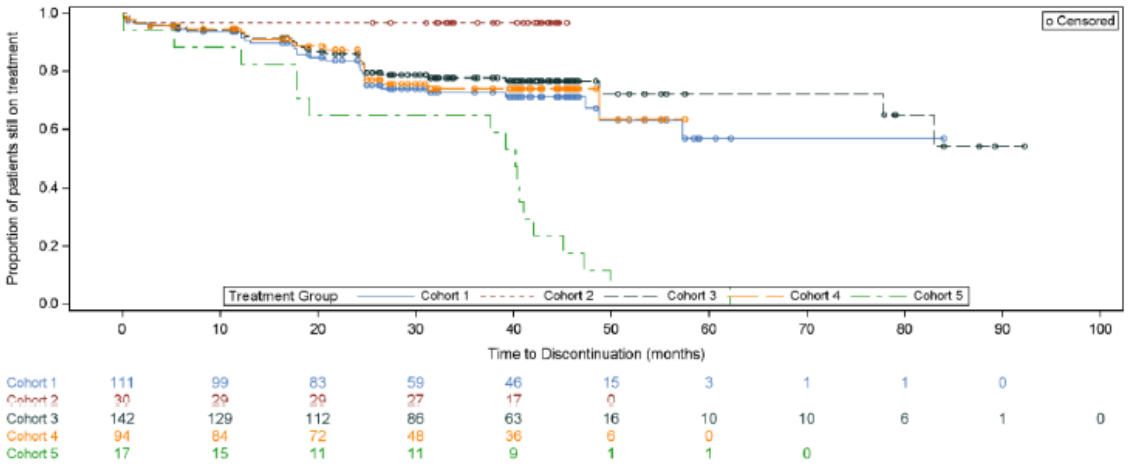
EOW: every other week; E4W: every 4 weeks; ERT: enzyme replacement therapy; PRX-102: pegunigalsidase alfa
* Patients who were treated with 0.2mg/kg or 2mg/kg in studies -F01 and -F02 and switched to 1mg/kg in study -F03 were included in Cohort 1 only from their time of 1st dose of 1mg/kg."

2.6.8.1. Patient exposure

As of 12 October 2021, 142 patients (94 male and 48 female) have been treated with pegunigalsidase alfa at any dose or posology in clinical studies. See

Figure 19.

Figure 19 Time of Treatment with Pegunigalsidase Alfa by Cohort



Cohort 1	111	99	83	59	46	15	3	1	1	0
Cohort 2	30	29	29	27	17	0				
Cohort 3	142	129	112	86	63	16	10	10	6	1	0
Cohort 4	94	84	72	48	36	6	0			
Cohort 5	17	15	11	11	9	1	1	0		

Reference: ISS Figure 9.1

Note, numbers for Cohort 5 go down to zero as only naïve patients from studies PB-102-F01/F02/F03 are included, not patients from the corresponding extension study PB-102-F60. All 10 completers of Study PB-102-F03 continued to study -F60 after at least 36 months in study -F03.

	Cohort Description	Studies Included in the Cohort:PB-102-
Cohort 1	1 mg/kg every 2 weeks (EOW), all studies	F01, F02, F03, F20 (PRX-102 arm), F30, F60
Cohort 2	Switchers from other ERTs to 2 mg/kg every 4 weeks (E4W)	F50, F51
Cohort 3	Any dose and frequency	F01, F02, F03, F20 (PRX-102 arm), F30, F60, F50, F51
Cohort 4	Switchers from other ERTs to 1 mg/kg EOW	F20 (PRX-102 arm), F30, F60
Cohort 5	Naïve patients receiving 1 mg/kg EOW	F01, F02, F03

PRX-102 = pegunigalsidase alfa

Overall, cumulative exposure amounted to 4874.71 patient months (about 406 patient years in Cohort 3). Maximum individual exposure was 91 months; ISS Table 5.1.3).

Most of the exposure was from treatment with 1 mg/kg pegunigalsidase alfa EOW (Cohort 1: 3519.77 patient months; about 293.3 patient years). Exposure to the alternative dose of 2 mg/kg E4W, albeit lower, was still substantial with 1110.01 patient months in Cohort 2 (about 92.5 patient years).

As the main study F20 provides a comparative arm, patient exposure for this study in

Table 56.

Table 56 Summary of exposure – Safety set (final report)

	PRX-102 N=52	Agalsidase Beta N=25
Cumulative Exposure (Months)	1176.2	596.4
Exposure (Months):		
Mean (SE)	22.62 (0.72)	23.86 (0.27)
Median (Min; Max)	23.95 (0.9 ; 27.4)	23.95 (17.7 ; 26.0)

Notes:

- Cumulative Exposure (months) is the sum of exposure over all subjects.
- Exposure could have been prolonged due to COVID-19 restrictions.

Source: Table 14.3.1

Home Infusion

Table 57 presents the infusion received across the different settings.

Table 57 Number of Pegunigalsidase Alfa Infusions Received, Overall, at Home, or on Site

Number of infusions[a]	Overall	Gender		ADA status[b]		
Cohort 1 (1 mg/kg pegunigalsidase alfa EOW)						
	N=111	**Male N=70**	**Female N=41**	**Positive N=27**	**Induced N=17**	**Negative N=63**
Overall Cumulative number	7579	4660	2919	1921	1520	4133
Individual exposure Mean number (SD)	68.3 (37.0)	66.6 (38.5)	71.2 (34.6)	71.1 (34.2)	89.4 (38.3)	65.6 (33.7)
Median number	66.0	61.0	69.0	64.0	94.0	60.0
Range (min, max)	1; 180	1; 180	2; 127	2; 127	1; 180	5; 134
At home	**N=72**	**N=44**	**N=28**	**N=18**	**N=9**	**N=45**
Cumulative number	3481	2083	1398	842	524	2115
Individual exposure Mean number (SD)	48.3 (26.6)	47.3 (27.9)	49.9 (24.7)	46.8 (29.8)	58.2 (28.9)	47.0 (24.9)
On site	**N=111**	**N=70**	**N=41**	**N=27**	**N=17**	**N=63**
Cumulative number	4098	2577	1521	1079	996	2018
Individual exposure Mean number (SD)	36.9 (32.3)	36.8 (32.4)	37.1 (32.6)	40.0 (31.7)	58.6 (37.4)	32.0 (28.9)
Cohort 2 (2 mg/kg pegunigalsidase alfa E4W)						
	N=30	**Male N=24**	**Female N=6**	**Positive N=10**	**Induced N=0**	**Negative N=20**
Overall Cumulative number	1271	1020	251	460	0	811
Individual exposure Mean number (SD)	42.4 (12.4)	42.5 (13.6)	41.8 (6.8)	46.0 (19.5)	-	40.6 (6.7)
Median number	45.0	45.0	41.5	46.5	-	40.0
Range (min, max)	1; 78	1; 78	35; 49	1; 78	-	28; 49
At home	**N=21**	**N=18**	**N=3**	**N=8**	**N=0**	**N=13**
Cumulative number	548	481	67	288	-	260
Individual exposure Mean number (SD)	26.1 (14.5)	26.7 (14.4)	22.3 (10.5)	36.0 (11.1)	-	20.0 (13.2)
On site	**N=30**	**N=24**	**N=6**	**N=10**	**N=0**	**N=20**
Cumulative number	723	539	184	172	-	551
Individual exposure Mean number (SD)	24.1 (14.0)	22.5 (13.0)	30.7 (17.1)	17.2 (10.4)	-	27.6 (14.6)

ADA: Anti-drug antibody; BL: Baseline; EOW: Every other week; E4W: Every 4 weeks; N: Number of patients in group; SD: Standard deviation.
The summary for infusions administered at home is based on patients who had at least one home infusion
a. Complete and partial infusions are included
b. Positive = ADA positive at BL; Negative = ADA negative at BL and all post-BL assessments; Induced = ADA negative at BL and at least 1 ADA positive post-BL. Please note information was not available for all patients and timepoints.
Reference: ISS Table 5.2.1 and Table 5.2.2

Patient Disposition

There were 8 withdrawals, either due to subject decision (8 patients overall) or TEAEs (8 patients overall, 4 of them with TEAEs considered related to the treatment- see further details in relevant section below). In the 4 patients with related TEAEs, withdrawal occurred early in the study: after the 1st infusion of study treatment for 3 patients and after 2nd infusion for 1 patient.

Demographics and Baseline Characteristics

The demographics and baseline characteristics of the study population in Cohort 1 (1 mg/kg cohort) and in Cohort 2 (2 mg/kg cohort) are presented below. See

Table 58.

Table 58 Demographics and Baseline Characteristics

		Overall	Gender		ADA status [b]		
Cohort 1 (1 mg/kg pegunigalsidase alfa EOW)							
		N=111	**Male N=70**	**Female N=41**	**Positive N=27**	**Induced N=17**	**Negative N=63**
Gender (n, %)	Male	70 (63.1%)	70 (100.0%)	0	26 (96.3%)	10 (58.8%)	31 (49.2%)
	Female	41 (36.9%)	0	41 (100.0%)	1 (3.7%)	7 (41.2%)	32 (50.8%)
Age (years)	Mean (SD)	43.4 (11.1)	42.3 (11.5)	45.2 (10.0)	43.0 (10.5)	46.1 (10.7)	43.4 (11.1)
Age range (years)		17; 60	17; 60	21; 60	20; 60	24; 59	17; 60
Age at diagnosis (years)	Mean (SD)	29.4 (13.2)	28.0 (13.6)	31.9 (12.2)	25.7 (13.4)	36.1 (11.4)	29.7 (13.1)
Race (n, %)	Asian	2 (1.8%)	2 (2.9%)	0	1 (3.7%)	0	1 (1.6%)
	Black or African American	6 (5.4%)	5 (7.1%)	1 (2.4%)	1 (3.7%)	0	5 (7.9%)
	White	102 (91.9%)	62 (88.6%)	40 (97.6%)	25 (92.6%)	16 (94.1%)	57 (90.5%)
	Other	1 (0.9%)	1 (1.4%)	0	0	1 (5.9%)	0
Previous ERT experience (n, %)	Naïve	17 (15.3%)	11 (15.7%)	6 (14.6%)	2 (7.4%)	5 (29.4%)	9 (14.3%)
	Experienced	94 (84.7%)	59 (84.3%)	35 (85.4%)	25 (92.6%)	12 (70.6%)	54 (85.7%)
	Switch from Fabrazyme [a]	*72 (76.6%)*	*44 (74.6%)*	*28 (80.0%)*	*23 (92.0%)*	*5 (41.7%)*	*43 (79.6%)*
	Switch from Replagal [a]	*22 (23.4%)*	*15 (25.4%)*	*7 (20.0%)*	*2 (8.0%)*	*7 (58.3%)*	*11 (20.4%)*
Last continuous ERT, duration (years) [c]	Mean (SD)	6.86 (4.07)	7.75 (4.43)	5.37 (2.89)	7.30 (5.30)	6.12 (1.70)	6.63 (3.82)
ADA status for pegunigalsidase alfa at baseline (n, %)	Positive	27 (24.3%)	26 (37.1%)	1 (2.4%)	27 (100.0%)	0	0
	Negative	84 (75.7%)	44 (62.9%)	40 (97.6%)	0	17 (100.0%)	63 (100.0%)
Cohort 2 (2 mg/kg pegunigalsidase alfa E4W)							
		N=30	**Male N=24**	**Female N=6**	**Positive N=10**	**Induced N=0**	**Negative N=20**
Gender (n, %)	Male	24 (80.0%)	24 (100.0%)	0	10 (100.0%)	0	14 (70.0%)
	Female	6 (20.0%)	0	6 (100.0%)	0	0	6 (30.0%)
Age (years)	Mean (SD)	40.5 (11.3)	39.3 (12.2)	45.2 (5.3)	33.2 (9.6)		44.1 (10.5)
Age range (years)		19; 58	19; 58	37; 52	20; 48		19; 58
Age at diagnosis (years)	Mean (SD)	26.3 (15.0)	24.2 (15.8)	34.8 (7.1)	15.6 (10.5)		31.7 (14.3)
Race (n, %)	White	30 (100.0%)	24 (100.0%)	6 (100.0%)	10 (100.0%)	0	20 (100.0%)

		Overall	Gender		ADA status [b]		
		N=111	Male N=70	Female N=41	Positive N=27	Induced N=17	Negative N=63
Previous ERT experience (n, %)	Naïve	0	0	0	0	0	0
	Experienced	30 (100.0%)	24 (100.0%)	6 (100.0%)	10 (100.0%)	0	20 (100.0%)
	Switch from Fabrazyme [a]	*23 (76.7%)*	*19 (79.2%)*	*4 (66.7%)*	*10 (100.0%)*	*0*	*13 (65.0%)*
	Switch from Replagal [a]	*7 (23.3%)*	*5 (20.8%)*	*2 (33.3%)*	*0*	*0*	*7 (35.0%)*
Last continuous ERT, duration (years) [c]	Mean (SD)	8.39 (4.82)	9.06 (5.01)	5.72 (2.94)	9.56 (5.11)		7.81 (4.69)
ADA status for pegunigalsidase alfa at baseline (n, %)	Positive	10 (33.3%)	10 (41.7%)	0	10 (100.0%)	0	0
	Negative	20 (66.7%)	14 (58.3%)	6 (100.0%)	0	0	20 (100.0%)

ADA: Anti-drug antibody; BL: Baseline; EOW: Every other week; E4W: Every 4 weeks; ERT: Enzyme replacement therapy; N: Number of patients in dose group; n (%): Percentage based on N; SD: Standard deviation.

a. Percentages based on number of patients with ERT experience.
b. Positive = ADA positive at BL; Negative = ADA negative at BL and all post-BL assessments, Induced = ADA negative at BL and at least 1 ADA positive post-BL. Please note information was not available for all patients at all timepoints.
c. Duration of last ERT treatment is calculated only for switchers and refers to patients who had several periods of treatment with ERT in the past.

Reference: ISS Table 2.1, Table 2.2, Table 3.1, and Table 3.2.

Comparative data are only available from the main Phase 3 study PB-102-F20 (pegunigalsidase alfa vs agalsidase beta). It is thus proposed to make a specific focus on results of this clinical trial allowing to contextualize pegunigalsidase alfa safety data. Please refer to the efficacy section for the baseline data on this study. Additional baseline characteristics relevant for the safety analysis are presented in Table 59 and

Table 60.

Table 59 Proportion of patients who used ACEI and/or ARB - Safety set (final report)

	PRX-102 N=52		Agalsidase Beta N=25	
ACEi				
Use at baseline	4	(7.7%)	6	(24.0%)
Use at both baseline and during the study	5	(9.6%)	5	(20.0%)
ARBs				
Use at baseline	20	(38.5%)	10	(40.0%)
Use at both baseline and during the study	19	(36.5%)	10	(40.0%)
Both ACEi and ARBs				
Use at baseline	2	(3.8%)	0	
Use at both baseline and during the study	4	(7.7%)	1	(4.0%)

Source: Table 14.3.9.2

Table 60 Proportion of patients who used infusion premedications - Safety set (final report)

Use of Premedication	PRX-102 N=52	Agalsidase Beta N=25
Baseline	n = 52	n = 25
	21 (40.4%)	16 (64.0%)
Week 12	n = 50	n = 25
	4 (8.0%)	5 (20.0%)
Week 52	n = 49	n = 25
	3 (6.1%)	4 (16.0%)
Week 104	n = 47	n = 24
	3 (6.4%)	3 (12.5%)

Source: Table 14.3.9.3

2.6.8.2. Adverse events

The reporting frequencies of the most important TEAE categories are provided in

Table *61* .Overall, 5 SAEs were related (3 switchers to 1 mg/kg E4W and 1 naive patient), all occurring in the 1 mg/kg E4W Cohorts.

Table 61 High-level Summary of TEAEs by Cohort

Event Category		Study Cohort				
		Cohort 1 (1 mg/kg PRX-102 EOW) N=111	Cohort 2 (2 mg/kg PRX-102 E4W) N=30	Cohort 3 (any PRX-102 posology) N=142	Cohort 4 (switchers to 1 mg/kg PRX-102 EOW) N=94	Cohort 5 (naïve patients receiving 1 mg/kg PRX-102 EOW) N=17
Any TEAE	Events (rate [a])	1426 (486.2)	339 (366.5)	1952 (480.5)	1146 (485.0)	260 (590.9)
	n (%)	104 (93.7%)	28 (93.3%)	133 (93.7%)	88 (93.6%)	16 (94.1%)
Related[b] TEAE	Events (rate [a])	115 (39.2)	46 (49.7)	188 (46.3)	78 (33.0)	36 (81.8)
	n (%)	47 (42.3%)	11 (36.7%)	61 (43.0%)	39 (41.5%)	7 (41.2%)
Serious TEAE	Events (rate [a])	57 (19.4)	8 (8.6)	65 (16.0)	52 (22.0)	4 (9.1)
	n (%)	36 (32.4%)	6 (20.0%)	42 (29.6%)	32 (34.0%)	3 (17.6%)
Related, serious TEAE	Events (rate [a])	5 (1.7)	0	5 (1.2)	4 (1.7)	1 (2.3)
	n (%)	5 (4.5%)	0	5 (3.5%)	4 (4.3%)	1 (5.9%)

EOW: Every other week; E4W: Every 4 weeks; N: Number of patients in cohort; n (%): number of patients with events, percentage based on N; PRX-102: Pegunigalsidase alfa; TEAE: treatment-emergent adverse event (i.e., starting after 1st infusion of pegunigalsidase alfa).

a. Rate is calculated per 100 patient years
b. Related if TEAE is reported as possibly, probably or definitely related to study treatment.

Reference: ISS Table 6.1.2.1 to Table 6.1.2.5.

Adverse Events

TEAEs were most often reported in the SOC Infections and Infestations, followed by Nervous System Disorders and Musculoskeletal and Connective Tissue Disorders in Cohort 1. The most commonly reported PTs in Cohort 1 were Nasopharyngitis, followed by Headache, Fatigue, Back pain, Diarrhoea, Cough, and Upper respiratory tract infection, all reported in >15% of patients.

Table 62 Frequently Reported TEAEs (Reported in ≥7.5% of Patients[a]) in Cohort 1 and Cohort 2 by System Organ Class and Preferred Term[b]

System Organ Class Preferred Term	Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111		Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30	
	Number (%) Patients	Number (rate [c]) of Events	Number (%) of Patients	Number (rate [c]) of Events
At least one TEAE	*104 (93.7%)*	*1426 (486.2)*	*28 (93.3%)*	*339 (366.5)*
Infections and infestations	**77 (69.4%)**	**253 (86.3)**	**22 (73.3%)**	**74 (80.0)**
Nasopharyngitis	25 (22.5%)	42 (14.3)	8 (26.7%)	13 (14.1)
Upper respiratory tract infection	16 (14.4%)	25 (8.5)	5 (16.7%)	7 (7.6)
Corona virus infection	11 (9.9%)	11 (3.8)	3 (10.0%)	4 (4.3)
Sinusitis	10 (9.0%)	13 (4.4)	5 (16.7%)	6 (6.5)
Urinary tract infection	10 (9.0%)	11 (3.8)	1 (3.3%)	1 (1.1)
Bronchitis	9 (8.1%)	11 (3.8)	1 (3.3%)	1 (1.1)
Viral infection	6 (5.4%)	6 (2.0)	4 (13.3%)	4 (4.3)
Gastroenteritis	5 (4.5%)	6 (2.0)	3 (10.0%)	7 (7.6)
Nervous system disorders	**55 (49.5%)**	**142 (48.4)**	**16 (53.3%)**	**26 (28.1)**
Headache	24 (21.6%)	44 (15.0)	3 (10.0%)	4 (4.3)
Dizziness	15 (13.5%)	24 (8.2)	0	0
Paraesthesia	9 (8.1%)	11 (3.8)	4 (13.3%)	4 (4.3)
Neuralgia	5 (4.5%)	6 (2.0)	4 (13.3%)	4 (4.3)
Neuropathy peripheral	3 (2.7%)	3 (1.0)	3 (10.0%)	3 (3.2)
Musculoskeletal and connective tissue disorders	**55 (49.5%)**	**139 (47.4)**	**13 (43.3%)**	**23 (24.9)**
Back pain	21 (18.9%)	26 (8.9)	4 (13.3%)	5 (5.4)
Arthralgia	16 (14.4%)	19 (6.5)	2 (6.7%)	2 (2.2)
Pain in extremity	16 (14.4%)	26 (8.9)	5 (16.7%)	6 (6.5)
Muscle spasms	10 (9.0%)	12 (4.1)	1 (3.3%)	1 (1.1)
Musculoskeletal pain	9 (8.1%)	9 (3.1)	0	0
Gastrointestinal disorders	**54 (48.6%)**	**144 (49.1)**	**11 (36.7%)**	**41 (44.3)**
Diarrhoea	21 (18.9%)	33 (11.3)	3 (10.0%)	5 (5.4)
Nausea	14 (12.6%)	18 (6.1)	5 (16.7%)	5 (5.4)
Abdominal pain	13 (11.7%)	21 (7.2)	2 (6.7%)	2 (2.2)
Vomiting	13 (11.7%)	17 (5.8)	4 (13.3%)	4 (4.3)
Constipation	2 (1.8%)	2 (0.7)	3 (10.0%)	3 (3.2)

Related Adverse Events

The reported events were mostly considered IRRs, i.e., reported during infusion or shortly afterwards. Related events were generally mild or moderate in intensity, with 6 events in 6 (5.4%) patients considered severe in Cohort 1, resolved on continued treatment and only in 5 (4.5%) patients were serious (Bronchospasm, Hypersensitivity, and Chills in 1 patient each, and Type I hypersensitivity in 2 patients). 4 among the 5 related Serious Adverse Events (SAEs) were indicative of hypersensitivity reactions. See

Table 63.

Table 63 Patients with Frequently Reported Related TEAEs (in more than 1 Patient Overall) in Cohort 1 and Cohort 2, by System Organ Class and Preferred Term

System Organ Class Preferred Term	Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111		Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30		PB-102-F20 (1 mg/kg pegunigalsidase alfa EOW) N=52		PB-102-F20 (1 mg/kg Fabrazyme EOW) N=25	
	Number (%) Patients	Number (rate[b]) of Events	Number (%) Patients	Number (rate [b]) of Events	Number (%) Patients	Number (rate[b]) of Events	Number (%) Patients	Number (rate[b]) of Events
At least one related TEAE	*47 (42.3%)*	*115 (39.2)*	*11 (36.7%)*	*46 (49.7)*	*21 (40.4%)*	*42 (42.9)*	*11 (44.0%)*	*76 (152.9)*
Gastrointestinal disorders	**13 (11.7%)**	**21 (7.2)**	**3 (10.0%)**	**4 (4.3)**	**5 (9.6%)**	**11 (11.2)**	**1 (4.0%)**	**4 (8.0)**
Nausea	6 (5.4%)	7 (2.4)	1 (3.3%)	1 (1.1)	2 (3.8%)	3 (3.1)	1 (4.0%)	1 (2.0)
Abdominal discomfort	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Abdominal pain	2 (1.8%)	2 (0.7)	1 (3.3%)	1 (1.1)	1 (1.9%)	1 (1.0)	0	0
Diarrhoea	2 (1.8%)	2 (0.7)	1 (3.3%)	1 (1.1)	1 (1.9%)	1 (1.0)	1 (4.0%)	1 (2.0)
Vomiting	2 (1.8%)	4 (1.4)	1 (3.3%)	1 (1.1)	2 (3.8%)	4 (4.1)	0	0
General disorders and administration site conditions	**12 (10.8%)**	**22 (7.5)**	**2 (6.7%)**	**10 (10.8)**	**8 (15.4%)**	**10 (10.2)**	**5 (20.0%)**	**15 (30.2)**
Fatigue	5 (4.5%)	9 (3.1)	1 (3.3%)	1 (1.1)	3 (5.8%)	3 (3.1)	2 (8.0%)	3 (6.0)
Chills	3 (2.7%)	3 (1.0)	0	0	2 (3.8%)	2 (2.0)	1 (4.0%)	1 (2.0)
Chest discomfort	1 (0.9%)	1 (0.3)	1 (3.3%)	1 (1.1)	0	0	2 (8.0%)	3 (6.0)
Pain	1 (0.9%)	1 (0.3)	1 (3.3%)	6 (6.5)	0	0	1 (4.0%)	3 (6.0)
Nervous system disorders	**11 (9.9%)**	**14 (4.8)**	**2 (6.7%)**	**3 (3.2)**	*8 (15.4%)*	*8 (8.2)*	*4 (16.0%)*	*4 (8.0)*
Dizziness	4 (3.6%)	5 (1.7)	0	0	1 (1.9%)	1 (1.0)	0	0
Headache	3 (2.7%)	3 (1.0)	1 (3.3%)	1 (1.1)	2 (3.8%)	2 (2.0	2 (8.0%)	2 (4.0)
Paraesthesia	2 (1.8%)	2 (0.7)	1 (3.3%)	1 (1.1)	2 (3.8%)	2 (2.0)	1 (4.0%)	1 (2.0)
Skin and subcutaneous tissue disorders	**6 (5.4%)**	**10 (3.4)**	**1 (3.3%)**	**1 (1.1)**	**1 (1.9%)**	**1 (1.0)**	**5 (20.0%)**	**37 (74.4)**
Pruritus	3 (2.7%)	3 (1.0)	0	0	0	0	3 (12.0%)	23 (46.3)
Rash maculo-papular	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Rash pruritic	2 (1.8%)	2 (0.7)	0	0	1 (1.9%)	1 (1.0)	1 (4.0%)	1 (2.0)
Erythema	1 (0.9%)	1 (0.3)	1 (3.3%)	1 (1.1)	0	0	1 (4.0%)	4 (8.0)
Immune system disorders	**6 (5.4%)**	**9 (3.1)**	**1 (3.3%)**	**1 (1.1)**	**2 (3.8%)**	**3 (3.1)**	**1 (4.0%)**	**1 (2.0)**
Hypersensitivity	3 (2.7%)	3 (1.0)	1 (3.3%)	1 (1.1)	2 (3.8%)	2 (2.0)	1 (4.0%)	1 (2.0)
Drug hypersensitivity	2 (1.8%)	4 (1.4)	0	0	1 (1.9%)	1 (1.0)	0	0
Type I hypersensitivity	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Injury, poisoning and procedural complications	**5 (4.5%)**	**7 (2.4)**	**4 (13.3%)**	**18 (19.5)**	**2 (3.8%)**	**2 (2.0)**	**1 (4.0%)**	**5 (10.1)**
Infusion related reaction[c]	5 (4.5%)	7 (2.4)	4 (13.3%)	18 (19.5)	2 (3.8%)	2 (2.0)	1 (4.0%)	5 (10.1)
Respiratory, thoracic and mediastinal disorders	**4 (3.6%)**	**10 (3.4)**	**0**	**0**	**1 (1.9%)**	**1 (1.0)**	**1 (4.0%)**	**2 (4.0)**
Musculoskeletal and connective tissue disorders	**3 (2.7%)**	**3 (1.0)**	**3 (10.0%)**	**3 (3.2)**	**0 (0.0%)**	**0**	**2 (8.0%)**	**4 (8.0)**
Arthralgia	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Pain in extremity	0	0	2 (6.7%)	2 (2.2)	0	0	0	0
Psychiatric disorders	**3 (2.7%)**	**3 (1.0)**	**0**	**0**	**1 (1.9%)**	**1 (1.0)**	**1 (4.0%)**	**1 (2.0)**
Ear and labyrinth disorders	**2 (1.8%)**	**2 (0.7)**	**0**	**0**	**0**	**0**	**0**	**0**
Vertigo	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Cardiac disorders	**2 (1.8%)**	**2 (0.7)**	**2 (6.7%)**	**2 (2.2)**	**0**	**0**	**0**	**0**
Supraventricular extrasystoles	2 (1.8%)	2 (0.7)	0	0	0	0	0	0
Vascular disorders	**3 (2.7%)**	**5 (1.7)**	**1 (3.3%)**	**1 (1.1)**	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Investigations	**3 (2.7%)**	**3 (1.0)**	**2 (6.7%)**	**3 (3.2)**	**1 (1.9%)**	**1 (1.0)**	**2 (8.0%)**	**3 (6.0)**
Renal and urinary disorders	**2 (1.8%)**	**3 (1.0)**	**0**	**0**	**1 (1.9%)**	**2 (2.0)**	**0**	**0**

EOW: Every other week; E4W: Every 4 weeks; N: Number of patients in cohort; (%): percentages based on N; TEAE: treatment-emergent adverse event.

a. Medical Dictionary for Regulatory Activities (MedDRA) Version 19.0.
b. Event rate is calculated per 100 patients-years of treatment.
c. This is a MedDRA Preferred Term and different from the IRRs defined in 2.7.4.3.5.1.

Reference: ISS Table 6.2.1.2; PB-102-F20 CSR Table 14.3.3.5

Data from the main study PB-102- F20 are presented in Table 64 and

Table 65.

Table 64 Summary of treatment-emergent adverse events by gender – Safety set (final report)

	PRX-102 Gender		Agalsidase beta Gender	
	Male N=29	**Female N=23**	**Male N=18**	**Female N=7**
All adverse events				
Number of any TEAE (rate)[1]	294 (545.32)	267 (605.42)	329 (922.09)	77 (549.09)
Number of subjects with any TEAE (n (%))	25 (86.2%)	22 (95.7%)	18 (100.0%)	6 (85.7%)
Number of severe TEAEs (rate)[1]	23 (42.66)	3 (6.80)	19 (53.25)	0
Number of subjects with severe[2] TEAEs (n (%))	12 (41.4%)	3 (13.0%)	7 (38.9%)	0
Number of serious TEAEs (rate)[1]	13 (24.11)	1 (2.27)	11 (30.83)	0
Number of subjects with serious TEAEs (n (%))	7 (24.1%)	1 (4.3%)	6 (33.3%)	0
Related[3] adverse events only				
Number of related TEAEs (rate)[1]	33 (61.21)	9 (20.41)	55 (154.15)	21 (149.75)
Number of subjects with related TEAEs (n (%))	15 (51.7%)	6 (26.1%)	9 (50.0%)	2 (28.6%)
Number of related severe[2] TEAEs (rate)[1]	2 (3.71)	0	1 (2.80)	0
Number of subjects with related severe TEAEs (n (%))	2 (6.9%)	0	1 (5.6%)	0
Number of related serious TEAEs (rate)[1]	1 (1.85)	0	0	0
Number of subjects with related serious TEAEs (n (%))	1 (3.4%)	0	0	0

[1] Rate is calculated as the adjusted number of events per 100 years of exposure.

[2] Events classified as "Very Severe" per CTCAE severity in the eCRF are included in the category "Severe".

[3] A TEAE was defined as related if was reported as possibly, probably, or definitely related to study drug.

Source: Table 14.3.3.1.1

Table 65 Summary of treatment-emergent adverse events by ADA status – Safety Population (final report)

	PRX-102 ADA status		Agalsidase beta ADA status	
	Negative N=34	**Positive N=18**	**Negative N=17**	**Positive N=8**
All adverse events				
Number of any TEAE (rate)[1]	382 (589.96)	179 (538.10)	218 (651.22)	188 (1158.5)
Number of subjects with any TEAE (n (%))	31 (91.2%)	16 (88.9%)	16 (94.1%)	8 (100.0%)
Number of severe[2] TEAEs (rate)[1]	15 (23.17)	11 (33.07)	12 (35.85)	7 (43.14)
Number of subjects with severe[2] TEAEs (n (%))	10 (29.4%)	5 (27.8%)	5 (29.4%)	2 (25.0%)
Number of serious TEAEs (rate)[1]	5 (7.72)	9 (27.06)	6 (17.92)	5 (30.81)
Number of subjects with serious TEAEs (n (%))	4 (11.8%)	4 (22.2%)	3 (17.6%)	3 (37.5%)
Related[3] adverse events only				
Number of related TEAEs (rate)[1]	13 (20.08)	29 (87.18)	28 (83.64)	48 (295.80)
Number of subjects with related TEAEs (n (%))	10 (29.4%)	11 (61.1%)	4 (23.5%)	7 (87.5%)
Number of related severe[2] TEAEs (rate)[1]	0	2 (6.01)	0	1 (6.16)
Number of subjects with related severe[2] TEAEs (n (%))	0	2 (11.1%)	0	1 (12.5%)
Number of related serious TEAEs (rate)[1]	0	1 (3.01)	0	0
Number of subjects with related serious TEAEs (n (%))	0	1 (5.6%)	0	0

[1] Rate is calculated as the adjusted number of events per 100 years of exposure.

[2] Events classified as "Very Severe" per CTCAE severity in the eCRF are included in the category "Severe".

[3] A TEAE was defined as related if was reported as possibly, probably, or definitely related to study drug.

Source: Table 14.3.3.1.2

The SOC in which with the more reported TEAEs are presented Table 66.

Table 66 Summary of system organ class disorders seen in >25% of patients in a group – Safety set

System Organ Class	PRX-102 N=52		Agalsidase Beta N=25	
	Number (%) of Subjects	Number of Events (rate)[1]	Number (%) of Subjects	Number of Events (rate)[1]
Infections and infestations	38 (73.1%)	105 (107.1)	16 (64.0%)	74 (148.9)
Nervous system disorders	29 (55.8%)	62 (63.3)	14 (56.0%)	32 (64.4)
Musculoskeletal and connective tissue disorders	28 (53.8%)	58 (59.2)	11 (44.0%)	31 (62.4)
Gastrointestinal disorders	24 (46.2%)	54 (55.1)	17 (68.0%)	47 (94.6)
General disorders and administration site conditions	22 (42.3%)	48 (49.0)	14 (56.0%)	38 (76.5)
Respiratory, thoracic and mediastinal disorders	19 (36.5%)	34 (34.7)	13 (52.0%)	29 (58.3)
Investigations	16 (30.8%)	43 (43.9)	8 (32.0%)	14 (28.2)
Skin and subcutaneous tissue disorders	17 (32.7%)	19 (19.4)	9 (36.0%)	48 (96.6)
Cardiac disorders	16 (30.8%)	25 (25.5)	10 (40.0%)	17 (34.2)
Injury, poisoning and procedural complications	15 (28.8%)	22 (22.4)	12 (48.0%)	30 (60.4)

[1] Rate is calculated as the adjusted number of events per 100 years of exposure.

Source: Table 14.3.3.3

In the pegunigalsidase alfa arm, 7 severe events in 5 patients were considered serious: aortic stenosis, bronchitis, acute kidney injury and dehydration, atrioventricular block second degree and protein-losing gastroenteropathy, and hypersensitivity. The event of hypersensitivity occurred during the first infusion, and was considered related to study product. (The patient remained in the study but experienced another event of drug hypersensitivity during the second infusion, this time of moderate intensity, and then withdrew from the study.) The time to onset of the other severe SAEs ranged from 113 days to 516 days.

The event of protein-losing gastroenteropathy was still ongoing as of the cut-off date for the interim analysis (no update was provided in the final analysis). All other events of severe intensity were non-serious, were considered unrelated to study product, and resolved, including the serious event of hypersensitivity.

In the agalsidase beta arm, 8 severe events in 4 patients were considered serious: acute respiratory failure and altered state of consciousness, pneumonia and sepsis, ventricular tachycardia and suicidal ideation, and chronic obstructive pulmonary disease and chest pain. The time to onset of these events ranged from 354 days to 729 days. None were considered related to study product. As of the cut-off date, the event of sepsis was still ongoing; all other events had resolved.

The only TEAEs of severe intensity that were considered related to study product were the serious event of hypersensitivity, described above, and a non-serious renal event of glomerulonephritis membranoproliferative in a pegunigalsidase alfa patient, from which the patient was still recovering as of the cut-off date.

2.6.8.3. Serious adverse event/deaths/other significant events

Death

Three deaths are reported as follows:

- A 35-year old male patient in study PB-102-F03 presented with chronic obstructive pulmonary disease resulting in death after an exposure of 38.1 months;

- a 58 year old male patient experienced a cerebrovascular accident (stroke) resulting in death after 24 months of Fabrazyme treatment in Study PB-102-F20 followed by 17 months of treatment with pegunigalsidase alfa 1 mg/kg EOW in Study PB-102-F60.

- a 60-year old male patient experienced sudden death after 24 months of Fabrazyme treatment in Study PB-102-F20 followed by 20 months of treatment with pegunigalsidase alfa 1 mg/kg EOW in Study PB-102-F60.

Other Serious Adverse Events

SAEs reported in at least 2 patients in Cohort 1 and Cohort 2 are presented in Table 67.

Table 67 Patients with Frequently Reported Serious TEAEs (in More than 1 Patient Overall) in Cohort 1 and Cohort 2, by System Organ Class and Preferred Term

System Organ Class Preferred Term	Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111		Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30	
	Number (%) of Patients	Number (rate[b]) of Events	Number (%) of Patients	Number (rate[b]) of Events
At least one serious TEAE	*36 (32.4%)*	*57 (19.4)*	*6 (20.0%)*	*8 (8.6)*
Infections and infestations	**10 (9.0%)**	**10 (3.4)**	**1 (3.3%)**	**1 (1.1)**
Pneumonia	2 (1.8%)	2 (0.7)	0	0
Sepsis	2 (1.8%)	2 (0.7)	0	0
Arthritis bacterial	1 (0.9%)	1 (0.3)	0	0
Bronchitis	1 (0.9%)	1 (0.3)	0	0
Cellulitis	1 (0.9%)	1 (0.3)	0	0
Corona virus infection	1 (0.9%)	1 (0.3)	0	0
Infectious mononucleosis	1 (0.9%)	1 (0.3)	0	0
Urinary tract infection	1 (0.9%)	1 (0.3)	0	0
Peritonitis bacterial	0	0	1 (3.3%)	1 (1.1)
Cardiac disorders	**10 (9.0%)**	**11 (3.8)**	**0**	**0**
Acute myocardial infarction	2 (1.8%)	2 (0.7)	0	0
Angina pectoris	1 (0.9%)	1 (0.3)	0	0
Atrial fibrillation	1 (0.9%)	1 (0.3)	0	0
Atrial flutter	1 (0.9%)	1 (0.3)	0	0
Atrioventricular block second degree	1 (0.9%)	1 (0.3)	0	0
Bradycardia	1 (0.9%)	1 (0.3)	0	0
Cardiac failure	1 (0.9%)	1 (0.3)	0	0
Cardiac failure congestive	1 (0.9%)	1 (0.3)	0	0
Cardiovascular symptom	1 (0.9%)	1 (0.3)	0	0
Myocardial ischaemia	1 (0.9%)	1 (0.3)	0	0
Nervous system disorders	**6 (5.4%)**	**7 (2.4)**	**0**	**0**
Cerebrovascular accident	3 (2.7%)	4 (1.4)	0	0
Headache	1 (0.9%)	1 (0.3)	0	0
Loss of consciousness	1 (0.9%)	1 (0.3)	0	0
Syncope	1 (0.9%)	1 (0.3)	0	0
Respiratory, thoracic and mediastinal disorders	**5 (4.5%)**	**5 (1.7)**	**0**	**0**
Chronic obstructive pulmonary disease	2 (1.8%)	2 (0.7)	0	0
Bronchospasm	1 (0.9%)	1 (0.3)	0	0
Eosinophilic bronchitis	1 (0.9%)	1 (0.3)	0	0
Obstructive airways disorder	1 (0.9%)	1 (0.3)	0	0
Immune system disorders	**3 (2.7%)**	**3 (1.0)**	**0**	**0**
Type I hypersensitivity	2 (1.8%)	2 (0.7)	0	0
Hypersensitivity	1 (0.9%)	1 (0.3)	0	0
Vascular disorders	**3 (2.7%)**	**3 (1.0)**	**0**	**0**
Aortic stenosis	2 (1.8%)	2 (0.7)	0	0
Venous thrombosis limb	1 (0.9%)	1 (0.3)	0	0

System Organ Class Preferred Term	Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111		Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30	
	Number (%) of Patients	Number (rate[b]) of Events	Number (%) of Patients	Number (rate[b]) of Events
Injury, poisoning and procedural complications	**5 (4.5%)**	**5 (1.7)**	**2 (6.7%)**	**2 (2.2)**
Clavicle fracture	1 (0.9%)	1 (0.3)	0	0
Contusion	1 (0.9%)	1 (0.3)	0	0
Femur fracture	1 (0.9%)	1 (0.3)	0	0
Tendon rupture	1 (0.9%)	1 (0.3)	0	0
Vaccination complication	1 (0.9%)	1 (0.3)	0	0
Overdose	0	0	1 (3.3%)	1 (1.1)
Road traffic accident	0	0	1 (3.3%)	1 (1.1)
Surgical and medical procedures	**3 (2.7%)**	**3 (1.0)**	**0**	**0**
Implantable cardiac monitor insertion	1 (0.9%)	1 (0.3)	0	0
Medical device battery replacement	1 (0.9%)	1 (0.3)	0	0
Nephrectomy	1 (0.9%)	1 (0.3)	0	0
General disorders and administration site conditions	**3 (2.7%)**	**3 (1.0)**	**1 (3.3%)**	**2 (2.2)**
Chills	1 (0.9%)	1 (0.3)	0	0
Hypothermia	1 (0.9%)	1 (0.3)	0	0
Sudden death	1 (0.9%)	1 (0.3)	0	0
Pain	0	0	1 (3.3%)	1 (1.1)
Pyrexia	0	0	1 (3.3%)	1 (1.1)
Investigations	**2 (1.8%)**	**2 (0.7)**	**0**	**0**
Electrocardiogram ST segment elevation	1 (0.9%)	1 (0.3)	0	0
Hepatic enzyme increased	1 (0.9%)	1 (0.3)	0	0
Gastrointestinal disorders	**2 (1.8%)**	**2 (0.7)**	**2 (6.7%)**	**2 (2.2)**
Pneumoperitoneum	1 (0.9%)	1 (0.3)	0	0
Protein-losing gastroenteropathy	1 (0.9%)	1 (0.3)	0	0
Hypoaesthesia oral	0	0	1 (3.3%)	1 (1.1)
Ileus	0	0	1 (3.3%)	1 (1.1)
Musculoskeletal and connective tissue disorders	**1 (0.9%)**	**1 (0.3)**	**1 (3.3%)**	**1 (1.1)**
Soft tissue mass	1 (0.9%)	1 (0.3)	0	0
Musculoskeletal chest pain	0	0	1 (3.3%)	1 (1.1)
Metabolism and nutrition disorders	**1 (0.9%)**	**1 (0.3)**	**0**	**0**
Dehydration	1 (0.9%)	1 (0.3)	0	0
Renal and urinary disorders	**1 (0.9%)**	**1 (0.3)**	**0**	**0**
Acute kidney injury	1 (0.9%)	1 (0.3)	0	0

EOW: Every other week; E4W: Every 4 weeks; N: Number of patients in cohort; (%): percentages based on N; TEAE: treatment-emergent adverse event.

a. Medical Dictionary for Regulatory Activities (MedDRA) Version 19.0.
b. Event rate is calculated per 100 patients-years of treatment.

Reference: ISS Table 6.2.1.3

Hypersensitivity related events

Related SAEs were reported for 5 patients, all in Cohort 1. Among them, 4 were indicative of hypersensitivity reactions: a case of severe Bronchospasm (IgE mediated hypersensitivity reaction) in study PB-102-F01, 2 cases of Type I hypersensitivity in study PB-102-F30, and a case of hypersensitivity in study PB-102-F20, which all occurred during the first infusion of study treatment.

Renal events

A related severe case of membranoproliferative glomerulonephritis was reported for a patient treated with pegunigalsidase alfa in Study PB-102-F20. To investigate the patient's persistent proteinuria, a kidney biopsy had been performed which confirmed immune complex mediated membranoproliferative glomerulonephritis with subendothelial IgG deposits as well as lambda and kappa immunoglobulin deposits. Immune complexes found in capillary and endothelial cells tested positive for alpha galactosidase. This severe TEAE led to interruption of treatment but not to study discontinuation. This patient experienced 14 other AEs, including a moderate event of proteinuria on Day 550.

Infusion Related Reactions

Infusion-related reactions (IRRs) were defined as TEAEs that occurred during an infusion or within 2 hours after its completion, and whose causality was assessed as definitely, probably, or possibly related to study treatment. IRRs do not include injection site reactions (ISRs), which are considered to be related to the procedure rather than the study drug.

The reporting frequencies for any IRR and serious IRRs are provided in Table 68,

Table 69 and

Table 70.

Table 68 High-level Summary of IRRs by Cohort

Event Category		Study Cohort				
		Cohort 1 (1 mg/kg PRX-102 EOW) N=111	Cohort 2 (2 mg/kg PRX-102 E4W) N=30	Cohort 3 (any PRX-102 posology) N=142	Cohort 4 (switchers to 1 mg/kg PRX-102 EOW) N=94	Cohort 5 (naïve patients with 1 mg/kg PRX-102 EOW) N=17
Any IRR	Events (rate[b])	52 (0.7)	38 (3.0)	103 (1.1)	35 (0.6)	17 (1.5)
	n (%)	26 (23.4%)	6 (20.0%)	35 (24.6%)	22 (23.4%)	4 (23.5%)
Serious IRR	Events (rate[b])	5 (0.1)	0	5 (0.1)	4 (0.1)	1 (0.1)
	n (%)	5 (4.5%)	0	5 (3.5%)	4 (4.3%)	1 (5.9%)

EOW: Every other week; E4W: Every four weeks; IRR: Infusion related reactions; N: number of patients in cohort; n (%): number of patients with events, percentage based on N; PRX-102: Peginugalsidase alfa; TEAE: treatment-emergent adverse event (i.e., occurring after start of 1st infusion of pegunigalsidase alfa).

a. IRRs were defined as those related TEAEs which occurred during the infusion or within 2 hours after the completion of the infusion and were related to study treatment rather than to procedures. The definition of IRRs is independent of the MedDRA preferred term "Infusion related reactions." See 2.7.4.1.4 for further details.
b. Rate is calculated per 100 infusions.

Reference: ISS Table 6.3.2.1.1 to 6.3.2.5.2.

Table 69 Summary of IRRs Overall, by Gender, and by ADA Status, for Cohort 1 and Cohort 2

Cohort 1 (1 mg/kg pegunigalsidase alfa EOW)							
		Overall	**Gender**		**ADA status [a]**		
		N=111	**Male, N=70**	**Female, N=41**	**Positive, N=27**	**Induced, N=17**	**Negative, N=63**
Any IRR	Events (rate[c])	52 (0.7)	37 (0.8)	15 (0.5)	16 (0.8)	16 (1.1)	18 (0.4)
	n (%)	26 (23.4%)	21 (30.0%)	5 (12.2%)	10 (37.0%)	6 (35.3%)	8 (12.7%)
Severe IRR	Events (rate[c])	5 (0.1)	5 (0.1)	0	2 (0.1)	1 (0.1)	0
	n (%)	5 (4.5%)	5 (7.1%)	0	2 (7.4%)	1 (5.9%)	0
Serious IRR	Events (rate[c])	5 (0.1)	5 (0.1)	0	2 (0.1)	1 (0.1)	0
	n (%)	5 (4.5%)	5 (7.1%)	0	2 (7.4%)	1 (5.9%)	0
IRR leading to study withdrawal	Events (rate[c])	4 (0.1)	4 (0.1)	0	1 (0.1)	1 (0.1)	0
	n (%)	4 (3.6%)	4 (5.7%)	0	1 (3.7%)	1 (5.9%)	0
Cohort 2 (2 mg/kg pegunigalsidase alfa E4W)							
		Overall	**Gender**		**ADA status [a]**		
		N=30	**Male, N=24**	**Female, N=6**	**Positive, N=10**	**Induced, N=0**	**Negative, N=20**
Any IRR	Events (rate[c])	38 (3.0)	38 (3.7)	0	35 (7.6)	0	3 (0.4)
	n (%)	6 (20.0%)	6 (25.0%)	0	5 (50.0%)	0	1 (5.0%)
Severe IRR	Events (rate[c])	0	0	0	0	0	0
	n (%)	0	0	0	0	0	0
Serious IRR	Events (rate[c])	0	0	0	0	0	0
	n (%)	0	0	0	0	0	0
IRR leading to study withdrawal	Events (rate[c])	0	0	0	0	0	0
	n (%)	0	0	0	0	0	0

EOW: Every other week; E4W: Every four weeks; IRR: Infusion related reaction; N: Number of patients in group; n (%): number of patients with events, percentage based on N; TEAE: treatment-emergent adverse event (i.e., starting after 1st infusion of pegunigalsidase alfa).

a. IRRs were defined as those related TEAEs which occurred during the infusion or within 2 hours after the completion of the infusion and were related to study treatment rather than to procedures. The definition of IRRs is independent of the MedDRA preferred term "Infusion related reactions." See 2.7.4.1.4 for further details.
b. Positive = ADA positive at BL; Negative = ADA negative at BL and all post-BL assessments, Induced = ADA negative at BL and at least 1 ADA positive post-BL; Please note information was not available for all patients and timepoints.
c. Rate is calculated per 100 infusions

Reference: ISS Table 6.3.2.1.1 to Table 6.3.2.2.2

Table 70 Summary of IRRs by Duration of Treatment, for Cohort 1 and Cohort 2

		Cohort 1 (1 mg/kg pegunigalsidase alfa EOW)			Cohort 2 (2 mg/kg pegunigalsidase alfa E4W)		
		Year 1 N=111	Year 2 N=94	Year 3+ N=70	Year 1 N=30	Year 2 N=29	Year 3+ N=29
Any IRR	Events (rate[c])	44 (1.7)	5 (0.2)	3 (0.1)	27 (6.8)	8 (2.0)	3 (0.6)
	n (%)	23 (20.7%)	2 (2.1%)	3 (4.3%)	5 (16.7%)	4 (13.8%)	3 (10.3%)
Serious IRR	Events (rate[c])	5 (0.2)	0	0	0	0	0
	n (%)	5 (4.5%)	0	0	0	0	0
IRR leading to study withdrawal	Events (rate[c])	4 (0.2)	0	0	0	0	0
	n (%)	4 (3.6%)	0	0	0	0	0

EOW: Every other week; E4W: Every four weeks; IRR: Infusion related reaction; N: Number of patients having received at least 1 infusion; n (%): number of patients with events, percentage based on N; TEAE: treatment-emergent adverse event.

a. IRR were defined as those related TEAEs which occurred during the infusion or within 2 hours after the completion of the infusion and were related to study treatment rather than to procedures. The definition of IRRs is independent of the MedDRA preferred term "Infusion related reactions." See 2.7.4.1.4 for further details.
b. The columns of Year 1, Year 2 and Year 3+ show the events, which occurred during 1st year of treatment, 2nd year of treatment or from the 3rd year
c. Rate is calculated per 100 infusions

Reference: ISS Table 6.3.4.1.1 to Table 6.3.4.2.2

In the main study P102-F20, no deaths were reported. Further data on serious or significant AE from the main studies are presented in Table 71, Table 72, Table 73 and Table 74.

Table 71 Summary of serious adverse events by system organ class – Safety set (final report)

System Organ Class Preferred Term	PRX-102; N=52		Agalsidase Beta; N=25	
	Number (%) of Subjects	Number of Events (rate)[1]	Number (%) of Subjects	Number of Events (rate)[1]
Any SAE	*8 (15.4%)*	*14 (14.3)*	*6 (24.0%)*	*11 (22.1)*
Cardiac disorders	**1 (1.9%)**	**1 (1.0)**	**3 (12.0%)**	**3 (6.0)**
Atrioventricular block second degree	1 (1.9%)	1 (1.0)	0	0
Atrial fibrillation	0	0	1 (4.0%)	1 (2.0)
Tachycardia	0	0	1 (4.0%)	1 (2.0)
Ventricular tachycardia	0	0	1 (4.0%)	1 (2.0)
Gastrointestinal disorders	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Protein-losing gastroenteropathy	1 (1.9%)	1 (1.0))	0	0
General disorders and administration site conditions	**1 (1.9%)**	**1 (1.0)**	**2 (8.0%)**	**2 (4.0)**
Hypothermia	1 (1.9%)	1 (1.0)	0	0
Chest pain	0	0	2 (8.0%)	2 (4.0)
Immune system disorders	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Hypersensitivity	1 (1.9%)	1 (1.0)	0	0
Infections and infestations	**1 (1.9%)**	**1 (1.0)**	**1 (4.0%)**	**2 (4.0)**
Bronchitis	1 (1.9%)	1 (1.0)	0	0
Pneumonia	0	0	1 (4.0%)	1 (2.0)
Sepsis	0	0	1 (4.0%)	1 (2.0)
Injury, poisoning and procedural compl.	**2 (3.8%)**	**2 (2.0)**	**0**	**0**
Contusion	1 (1.9%)	1 (1.0)	0	0
Femur fracture	1 (1.9%)	1 (1.0)	0	0
Investigations	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Hepatic enzyme increased	1 (1.9%)	1 (1.0))	0	0
Metabolism and nutritional disorders	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Dehydration	1 (1.9%)	1 (1.0))	0	0
Nervous system disorders	**0**	**0**	**1 (4.0%)**	**1 (2.0)**
Altered state of consciousness	0	0	1 (4.0%)	1 (2.0)
Psychiatric disorders	**0**	**0**	**1 (4.0%)**	**1 (2.0)**
Suicidal ideation	0	0	1 (4.0%)	1 (2.0)
Renal and urinary disorders	**1 (1.9%)**	**1 (1.0)**	**0**	**0**
Acute kidney injury	1 (1.9%)	1 (1.0))	0	0
Respiratory, thoracic and mediastinal dis.	**0**	**0**	**2 (8.0%)**	**2 (4.0)**
Acute respiratory failure	0	0	1 (4.0%)	1 (2.0)
Chronic obstructive pulmonary disease	0	0	1 (4.0%)	1 (2.0)
Surgical and medical procedures	**2 (3.8%)**	**2 (2.0)**	**0**	**0**
Medical device battery replacement	1 (1.9%)	1 (1.0)	0	0
Nephrectomy	1 (1.9%)	1 (1.0)	0	0
Vascular disorders	**2 (3.8%)**	**2 (2.0)**	**0**	**0**
Aortic stenosis	1 (1.9%)	1 (1.0)	0	0
Venous thrombosis limb	1 (1.9%)	1 (1.0)	0	0

[1] Rate is calculated as the adjusted number of events per 100 years of exposure

Source: Table 14.3.3.6

The only SAE that was considered related to study drug was the severe event of hypersensitivity in a patient in the pegunigalsidase alfa arm, described earlier.

Table 72 Summary of IRRs occurring within 2 hours of infusion - Safety set (final report)

	PRX-102 N=52			Agalsidase Beta N=25		
	Number of patients with at least 1 IRR n (%)	Number of infusions with IRR	Number of IRRs (rate) [1]	Number of patients with at least 1 IRR n (%)	Number of infusions with IRR	Number of IRRs (rate) [1]
Any IRR	11 (21.2%)	12	13 (0.50)	6 (24.0%)	40	51 (3.9)
Severe IRR	1 (1.9%)	1	1 (0.0)	0	0	0
Serious IRR	1 (1.9%)	1	1 (0.0)	0	0	0
IRR leading to withdrawal	1 (1.9%)	1	1 (0.0)	0	0	0

[1] Rate = adjusted rate of events per 100 infusions

Source: Table 14.3.3.8

Table 73 Summary of IRRs occurring within 2 hours of infusion, by subgroup – Safety Set (final report)

	PRX-102 N=52		Agalsidase Beta N=25	
Gender	**Male N=29**	**Female N=23**	**Male N=18**	**Female N=7**
Number of Events (rate)[1]	11 (0.8)	2 (0.2)	33 (3.5)	18 (4.9)
Number of Infusions	10	2	24	16
Number (%) of Subjects	9 (31.0%)	2 (8.7%)	5 (27.8%)	1 (14.3%)
ADA Status	**Negative N=34**	**Positive N=18**	**Negative N=17**	**Positive N=8**
Number of Events (rate)[1]	5 (0.3)	8 (0.9)	19 (2.2)	32 (7.5)
Number of Infusions	5	7	17	23
Number (%) of Subjects	5 (14.7%)	6 (33.3%)	2 (11.8%)	4 (50.0%)
FD Classification	**Classic N=27**	**Non-Classic N=25**	**Classic N=14**	**Non-Classic N=11**
Number of Events (rate)[1]	10 (0.8)	3 (0.2)	33 (4.5)	18 (3.1)
Number of Infusions	9	3	24	16
Number (%) of Subjects	8 (29.6%)	3 (12.0%)	5 (35.7%)	1 (9.1%)
Region	**US N=33**	**ex-US N=19**	**US N=18**	**ex-US N=7**
Number of Events (rate)[1]	9 (0.6)	4 (0.4)	32 (3.3)	19 (5.5)
Number of Infusions	8	4	28	12
Number (%) of Subjects	8 (24.2%)	3 (15.8%)	5 (27.8%)	1 (14.3%)

[1] Rate = adjusted rate of events per 100 infusions

Source: Table 14.3.3.8.1, Table 14.3.3.8.2, Table 14.3.3.8.3, Table 14.3.3.8.4

Table 74 Summary of IRRs occurring within 24 hours of infusion - Safety set (final report)

	PRX-102 N=52			Agalsidase Beta N=25		
	Number of patients with at least 1 IRR n (%)	Number of infusions with IRR	Number of IRRs (rate) [1]	Number of patients with at least 1 IRR n (%)	Number of infusions with IRR	Number of IRRs (rate) [1]
Any IRR	17 (32.7%)	24	28 (1.1)	8 (32.0%)	45	62 (4.8)
Severe IRR	1 (1.9%)	1	1 (0.0)	0	0	0
Serious IRR	1 (1.9%)	1	1 (0.0)	0	0	0
IRR leading to withdrawal	1 (1.9%)	1	1 (0.0)	0	0	0

[1] Rate = adjusted rate of events per 100 infusions

Source: Table 14.3.3.8a

A case of membranoproliferative glomerulonephritis has been also reported in pegunigalsidase alfa arm, but already displayed above.

2.6.8.4. Laboratory findings

In summary, a limited number of patients experienced deteriorations in laboratory parameters while treated with pegunigalsidase alfa. Only 3 related TEAEs in 2 patients based on laboratory parameter were reported; these were increased urine protein/creatinine ratio (UPCR) and increased white blood cell count in 1 patient in Study PB-102-F50, and hepatic enzyme increased in another patient also in Study PB-102-F50.

Electrocardiogram Data

As is expected in the Fabry Disease population [Namdar 2016], clinically significant changes in ECG during the study were infrequently documented on case report forms (CRFs) based on locally performed ECG procedures. In none of the studies were meaningful safety signals detected based on the assessment of ECG parameters.

No safety signals emerged from the cardiac MRI assessments in the studies.

Brain MRI

In studies PB-102-F01/F02/F03, PB-102-F20, and PB-102-F50, no evidence of stroke was detected in patients' MRIs of the brain at Baseline and throughout the study.

In study PB-102-F30, abnormal findings at baseline only were reported for 1 patient (with evidence of stroke) and abnormal findings at baseline and at Month 12 were reported for 1 further patient (evidence of stroke). No further abnormal findings were reported.

Vital Signs

In the PB-102-F01 and PB-102-F02 studies, the majority of the vital signs measurements (systolic and diastolic blood pressures, pulse rate, temperature, and respiration rate) were within normal range. One female patient in the 1.0 mg/kg treatment group experienced 3 possibly/probably treatment-related events of hypotension, 2 of which were IRRs (transient, and resolved the same day).

In the PB-102-F30 study, there were no notable changes in the vital sign variables in the Safety population during the study.

In the PB-102-F20 study and the PB-102-F50 study, there were no findings of note for vital signs.

Vital sign analyses are currently not available for the ongoing studies PB-102-F60 and PB-102-F51.

Physical Examination Findings

The most common abnormal physical examination results in the PB-102-F01 and PB-102-F02 studies at the end of study were in skin (0.2 mg/kg, 66.7%; 1.0 mg/kg, 83.3%; 2.0 mg/kg, 50.0% of patients) and neurological body systems (0.2 mg/kg, 16.7%; 1.0 mg/kg, 16.7%; 2.0 mg/kg, 25.0% of patients). In PB-102-F03 at Month 24 the most common abnormal physical examination results were in skin (63.6% of patients), neurological and musculoskeletal (each 27.3% of patients), and head, eyes, ears, nose, throat (HEENT) and cardiovascular body systems (each 18.2% of patients).

The most common abnormal physical examination results in PB-102-F30 at Month 12 were also in skin (45.0% of patients), cardiovascular (each 15.0% of patients) and HEENT (10.0% of patients) body systems.

The most common abnormal physical examination results in PB-102-F50 at Month 12 were also in skin (75% of patients) and HEENT (25%).

There were no findings of note for physical examinations in PB-102-F20.

Physical examination analyses are currently not available for the ongoing studies PB-102-F60 and PB-102-F51.

In study PB-102-F20, 6 different laboratory-related TEAEs were seen in at least 2 patients within pegunigalsidase alfa group, while in the agalsidase beta arm, the only one seen in more than a single patient was increased blood creatinine. No events were rated as serious; only one event, decreased glomerular filtration rate in a patient in the pegunigalsidase alfa arm (1.9%), was assessed as severe; and only one, a mild event of increased urine/albumin ratio in a patient in the agalsidase beta arm (4.0%), was considered possibly related to study treatment. See Table 75.

Table 75 Clinically significant laboratory adverse events seen in ≥2 patient in a treatment group – Safety set

	PRX-102 N=52		Agalsidase Beta N=25	
	Number (%) of patients	Number of events	Number (%) of patients	Number of events
Investigations	15 (28.8%)	44	8 (32.0%)	14
Blood creatinine increased	3 (5.8%)	7	4 (16.0%)	5
Urine protein/creatinine ratio increased	3 (5.8%)	5	0	0
Creatinine urine increased	2 (3.8%)	3	0	0
Cystatin C increased	2 (3.8%)	2	0	0
International normalised ratio increased	2 (3.8%)	2	0	0

Source: Table 14.3.3.3

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

Intrinsic Factors

Subgroup analyses of safety data by age, race, or ethnic group were not conducted. Considering the overall small sample size, subgroups would have limited numbers of patients and it was expected that results would be difficult to interpret.

An exposure adjusted subgroup analysis of TEAEs by gender suggested a trend for higher reporting rates for any, related, severe, related severe, and serious TEAEs in male patients compared to female patients, as expected based on gender-specific differences in pathophysiology of the disease.

Extrinsic Factors

An exposure adjusted subgroup analysis of TEAEs by ADA status suggested a trend for higher reporting rates especially for related, severe, and serious TEAEs in ADA positive, followed by ADA induced patients, compared to ADA negative patients.

No safety analyses by renal impairment or hepatic impairment were performed. While study PB-102-F20 includes patients with renal impairment (screening eGFR 40 to 120 mL/min/1.73 m2 and linear negative slope of eGFR of at least 2 mL/min/1.73 m2), no data in patients with severe renal or severe hepatic impairment are available.

Use in Pregnancy and Lactation

There is limited available data on pegunigalsidase alfa use in pregnant women to determine the drug-associated risk.

There is no data on the presence of pegunigalsidase alfa in human milk, the effects on the breast fed infant or the effects on milk production.

In the current program for pegunigalsidase alfa, 2 pregnancies have been reported despite a requirement for contraception. One was reported in study PB-102-F03, the patient had normal ultrasound findings at week 13 of gestation but decided to terminate the pregnancy at week 14 for personal reasons. Another pregnancy in the ongoing Study PB-102-F60 was reported. The pregnancy occurred after more than 5 years of treatment with pegunigalsidase alfa in Studies PB-102-F01/02/03 and PB-102-F60 and led to discontinuation from the study. The pregnancy resulted in the birth of a healthy baby at gestational week 40.

Overdose

There have been no reports of overdose with pegunigalsidase alfa. In clinical trials, patients received doses up to 2.0 mg/kg body weight.

Withdrawal and Rebound

No evidence of withdrawal or rebound was observed.

Effects on Ability to Drive or Operate Machinery or Impairment of Mental Ability

No studies on the effects on the ability to drive and use machines have been performed.

2.6.8.7. Immunological events

Pre-existing and induced anti- pegunigalsidase alfa antibodies were observed in the clinical studies.

The development of anti-pegunigalsidase alfa IgG and neutralising anti-pegunigalsidase alfa ADA over time is presented in for Cohorts 1, 2 and 4. Immunogenicity assessments were performed following a multi-tiered approach, thus further ADA assessments (including tests for neutralising antibodies) were only performed in IgG-positive patients. See Table 76.

Table 76 Development of Anti-Pegunigalsidase Alfa Antibodies, for Cohort 1 and Cohort 2

		Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111	Cohort 4 (ERT switchers to 1 mg/kg pegunigalsidase alfa EOW); N=94	Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30
IgG				
Baseline	N*	111	94	30
	Positive; n (%)	27 (24.3%)	25 (26.6%)	10 (33.3%)
Between Baseline and Month 6	N*	106	90	29
	Positive[a]; n (%)	36 (34.0%)	31 (34.4%)	8 (27.6%)
Between Month 6 and Month 12	N*	100	85	29
	Positive[a]; n (%)	27 (27.0%)	25 (29.4%)	6 (20.7%)
Between Month 12 and Month 18	N*	83	68	12
	Positive[a]; n (%)	18 (21.7%)	17 (25.0%)	4 (33.3%)
Between Month 18 and Month 24	N*	67	55	3
	Positive[a]; n (%)	14 (20.9%)	14 (25.5%)	0
Between Month 24 and Month 30	N*	66	55	28
	Positive[a]; n (%)	14 (21.2%)	14 (25.5%)	5 (17.9%)
Between Month 30 and Month 36	N*	48	37	19
	Positive[a]; n (%)	9 (18.8%)	9 (24.3%)	3 (15.8%)
Between Month 36 and Month 42	N*	44	34	19
	Positive[a]; n (%)	6 (13.6%)	6 (17.6%)	5 (26.3%)
Between Month 42 and Month 48	N*	32	22	10
	Positive[a]; n (%)	6 (18.8%)	6 (27.3%)	2 (20.0%)
Between Month 48 and Month 54	N*	15	6	0
	Positive[a]; n (%)	3 (20.0%)	2 (33.3%)	
Between Month 54 and Month 60	N*	13	3	0
	Positive[a]; n (%)	2 (15.4%)	0	

		Cohort 1 (1 mg/kg pegunigalsidase alfa EOW) N=111	Cohort 4 (ERT switchers to 1 mg/kg pegunigalsidase alfa EOW); N=94	Cohort 2 (2 mg/kg pegunigalsidase alfa E4W) N=30
Between Month 60 and Month 66	N* Positive[a]; n (%)	9 1 (11.1%)	–	0
Between Month 66 and Month 72	N* Positive[a]; n (%)	8 1 (12.5%)	–	0
After Month 72	N* Positive[a]; n (%)	10 2 (20.0%)	–	0
At any post-baseline visit	*N** *Positive[a]; n (%)*	*107* *42 (39.3%)*	*91* *36 (39.6%)*	*29* *8 (27.6%)*
Neutralising ADA				
Baseline	N* Positive; n (%)	27 24 (88.9%)	25 24 (96.0%)	10 10 (100.0%)
Between Baseline and Month 6	N* Positive[a]; n (%)	35 25 (71.4%)	31 23 (74.2%)	8 7 (87.5%)
Between Month 6 and Month 12	N* Positive[a]; n (%)	27 17 (63.0%)	25 16 (64.0%)	6 5 (83.3%)
Between Month 12 and Month 18	N* Positive[a]; n (%)	18 13 (72.2%)	17 13 (76.5%)	4 2 (50.0%)
Between Month 18 and Month 24	N* Positive[a]; n (%)	14 11 (78.6%)	14 11 (78.6%)	0
Between Month 24 and Month 30	N* Positive[a]; n (%)	14 8 (57.1%)	14 8 (57.1%)	5 3 (60.0%)
Between Month 30 and Month 36	N* Positive[a]; n (%)	9 6 (66.7%)	9 6 (66.7%)	3 2 (66.7%)
>Month 36	N* Positive[a]; n (%)	11 4 (36.4%)	9 3 (33.3%)	5 3 (60.0%)
At any post-baseline visit	*N** *Positive[a]; n (%)*	*41* 26 (63.4%)	*36* *23 (63.9%)*	*8* *7 (87.5%)*
Treatment-emergent ADA[b]	N* Yes; n (%) Titre boosted; n (%) Treatment-induced; n (%) No; n (%)	107 25 (23.4%) 8 (7.5%) 17 (15.9%) 82 (76.6%)	91 19 (20.9%) 7 (7.7%) 12 (13.2%) 72 (79.1%)	29 2 (6.9%) 2 (6.9%) 0 27 (93.1%)

ADA: Anti-drug antibodies; EOW: Every other week; E4W: Every four weeks; ERT: Enzyme replacement therapy; IgE: Immunoglobulin E; IgG: Immunoglobulin G; N: Number of patients in Cohort; N*: Number of patients with data; n (%): Number of positive patients, percentage based on N*.

a. Positive in at least one post-baseline visit within the given time period

b. Patients are considered to be treatment-emergent ADA positive if they satisfy one of the following conditions: 1. Titre boosted: patients who were IgG positive at baseline and boosted post-treatment by at least four-fold, or
2.Treatment Induced: patients who were IgG-negative at baseline and positive in at least one timepoint post first infusion. Only patient with baseline evaluation and at least one post-baseline evaluation are considered for this variable.

Reference: ISS Table 7.1 and Table 7.1.2

2.6.8.8. Safety related to drug-drug interactions and other interactions

No specific drug interaction studies were performed with pegunigalsidase alfa.

2.6.8.9. Discontinuation due to adverse events

Data are presented in Table 77.

Table 77 Line Listing of TEAEs Leading to Withdrawal from the Study

Study	Patient	System Organ Class	Preferred Term	Serious	Severity	Causality	Cohort(s)
PB-102-F01	7[a]	Respiratory, thoracic and mediastinal disorders	Bronchospasm	Yes	Severe	Related	1/3/5
PB-102-F03	4	Respiratory, thoracic and mediastinal disorders	Chronic obstructive pulmonary disease	Yes	Severe	Unrelated	1/3/5
PB-102-F30	94[a]	Immune system disorders	Type I hypersensitivity	Yes	Severe	Related	1/3/4
PB-102-F30	110[a]	Immune system disorders	Type I hypersensitivity	Yes	Severe	Related	1/3/4
PB-102-F20	80	Renal and urinary disorders	End-stage renal disease	No	Severe	Unrelated	1/3/4
PB-102-F20	87[a]	Immune system disorders	Drug hypersensitivity	No	Moderate	Related	1/3/4
PB-102-F60	19	General disorders and administration site conditions	Sudden death	Yes	Severe	Unrelated	1/3/4
PB-102-F60	25	Nervous system disorders	Cerebrovascular accident	Yes	Severe	Unrelated	1/3/4

[a] see 2.7.4.3.4.1 for Patient Narratives
Reference: ISS Listing 10.8.4

In the main study PB-102-F20, two patients, both in the pegunigalsidase alfa arm, experienced TEAEs that led to withdrawal from the study; none in the agalsidase beta arm.

2.6.8.10. Post marketing experience

As claimed by the applicant, pegunigalsidase alfa has not been marketed in any country.

2.6.9. Discussion on clinical safety

The safety database includes patients with Fabry disease from two Phase 1/2 dose finding studies followed by an extension study, one main Phase 3 study and two supportive Phase 3 studies, one evaluating an alternative dose/regimen and one enrolling patients previously treated with Replagal. The safety data from all these clinical trials are pooled, which is understood given the low number of participants per study. Additionally, considering that there are no major discrepancies in selection criteria across the trials, the method is acceptable.

Comparative data are available for the 1 mg/kg dose EOW from the main Phase 3 study (PB-102-F20), a controlled trial using Fabrazyme (ERT administered EOW) as comparator. However, this is not the case for the 2 mg/kg dose E4W.

The number of patients naïve of any ERT treatments enrolled in the clinical program is quite low (i.e. n=17). Moreover, all those have been received 1 mg/kg dose EOW, none have been treated with the 2 mg/kg dose E4W.

More generally, the extent of the exposure for the high dose (n=30; 92.5 patients-year) is rather limited compared to the low dose (n=111; 293.3 patients-year). Likewise, long term data are scarce for these 2mg/kg dose E4W (n=17 at Month 30), although interim data from the extension study PB-102-F51 during the procedure.

In the overall safety population (n=142), TEAE were reported in 93.7% of the subjects with a rate of 480.5 events per 100 patient-years. Related TEAE were noted for 43.0 % of the patients with a rate of 46.3. Additionally, 29.6 % of the patients experienced serious TEAE (16.0 per 100 p-y), and 3.5% a related TEAE (1.2 per 100 p-y).

In cohort 1 (1 mg/kg dose EOW; naïve and switcher patients), the most commonly reported PTs were nasopharyngitis, followed by headache, fatigue, back pain, diarrhoea, cough, and upper respiratory tract infection, all reported in >15% of patients.

Regarding related TEAE, the PTs reported with the higher rate (in events per 100 patient-years) were Fatigue (3.1), Nausea (2.4), Infusion related reaction (2.4), Dizziness (1.7), Vomiting (1.4) and Hypersensitivity (1.4). Related TEAE were more reported in naïve patients (81.8 per 100 patient-years) than for the switcher patients (33.0). This suggests that the safety profile might be different in naïve of any other ERT treatments and non-naïve patients; and could be explained by first exposition to an ERT. However, data are too limited to conclude on difference between these 2 populations.

Regarding the high dose (2 mg/kg E4W; cohort 2), while the proportion of patients who experienced a related TEAE is slightly lower than for the low dose (36.7% vs 42.3%), the rate is rather higher with 49.7 events per 100 p-y comparing to 39.2. A substantial difference in disfavour of the high dose can be specifically observed for IRRs (16.7% of patients with a rate of 21.6 events/100 patients-year vs. 5.4% of patients with a rate of 2.7) and for pain (13.3% of patients with a rate of 18.4 vs. 4.5% of patients with 2.0). It is noted that difference in number of patients in each cohort (i.e. 1 and 2), with a limited number in the cohort 2, make it difficult to draw firm conclusion on the safety of the higher dose. However, at this stage, a dose-dependent effect cannot be fully excluded regarding the occurrence of IRR.

Regarding SAE, overall, the main represented SOC were infections and infestations (2 AE of pneumonia), respiratory, thoracic and mediastinal disorders (2 AE of chronic obstructive pulmonary disease), cardiac disorders, and injury poisoning and procedural complications. In cohort 1, among the SAE reported, 4 were assessed as related to the study drug (3.8 of the patients, 1.8 events per 100 p-year). No related SAE occurred in the cohort 2, however, the low extent of the exposure in this subset could be a limitation to observed events, in particular if not frequent.

In the main the Phase 3 study (PB-102-F20), the proportion of patients experiencing TEAE was overall similar across the treatments groups with 88.5% in the pegunigalsidase alfa arm and 96.0% in the Fabrazyme arm. However, the CHMP noted that the proportion of male was higher in the Fabrazyme arm than in the pegunigalsidase alfa arm (72.0% vs. 55.8%) at baseline.

Considering a difference in severity of the disease across gender (homozygote vs. heterozygote), a poorer safety profile can be expected for males. Thus, the imbalance introduces a bias in the interpretation of the data. It can also be observed that at baseline, a higher proportion of patients of the Fabrazyme arm was receiving premedication for use of Fabrazyme before enrolment, comparing to the pegunigalsidase alfa arm.

Therefore, this could also introduce a bias comparing safety profiles. However, based on the presented data by gender and status of pre-medication at baseline, no unexpected concern emerges. Nonetheless, through stratification, the number of patients, and thus of events, per strata is quite reduced limiting the interpretation. Due to these imbalances together with the low number of subjects enrolled, and consequently the limited number of events occurring during the trial,–it appears difficult to well characterize the differences in safety profile of pegunigalsidase alfa comparing to another ERT therapy.

Three (3) death events have been reported under pegunigalsidase alfa treatments, but not related to the treatment according to the Applicant.

IRR/hypersensitivity and Immunological Events

At initial submission, 4 serious Infusion Related Reactions (IRRs) related to hypersensitivity, were reported as SAE in Cohort 1, with a rate of 1.8 events per 100 p-year: 1 case of severe Bronchospasm (IgE mediated hypersensitivity reaction) and 3 cases of hypersensitivity (among them 2 are labelled as Type 1), which all occurred during the first infusion of study treatment. All the patients recovered and were discontinued from study. Additionally, all 4 serious cases of hypersensitivity reactions were reported in males and probably Ig-E mediated, occurred in the two hours (i.e. immediate). suggesting no delayed hypersensitivity reaction However, further data are awaited to further characterise these reactions.

Hypersensitivity Reactions (infusion related) is an important identified risk in the RMP to be further monitored through pharmacovigilance activities.

During the procedure, a fifth SAE was reported in Cohort 1 and relates to a case of Chills assessed as possibly related to Elfabrio and reported in a patient having received Fabrazyme in Study PB-102-F20 after 6 months of treatment with pegunigalsidase alfa in Study PB-102-F60. The event resolved, and the patient was re-administrated as planned, with premedication, and without experiencing further IRR.

IRRs (any severity) occurred respectively in 30.0% (21/70) and 25% (6/24) of males in cohorts 1 and 2 compared to 12.2% (5/41) and 0% (0/6) in females. This is nevertheless consistent with the difference in disease's severity across gender due to the hemizygosity and heterozygosity.

The IRR rate in naïve subset is 1.5 events per 100 p-year (cohort 5) compared to 0.6 events per 100 p-year in non-naïve (cohort 4). But, the low number of naive subjects (n=17) makes difficult the interpretation of this difference.

IRRs were also more frequent in patients with positive or induced ADA status. In cohort 1, 37.0% (10/27) of the patients with positive ADA and 35.3% (6/17) of the patients with induced ADA experienced IRRs while the proportion was 12.7% (8/63) in patients with negative ADA. Moreover, serious IRR occurred only in positive or induced ADA patients.

As reported earlier, a higher rate of IRR has been observed with the 2 mg/kg dose E4W. Additionally, while almost all IRR occurred during the Year 1 in cohort 1 (1 mg/kg dose) (5 cases in Year 2 with a rate of 0.2 per 100 infusions; 3 cases in Year 3 with a rate of 0.1 per 100 infusions), there is a substantial number of events reported during the Year 2 (8 cases with a rate of 2.0) and 3 (3 cases with a rate of 0.6) in the cohort 2 (2 mg/kg dose). This might also suggest that the decrease of hypersensitivity after long term exposure, is less reported with the higher dose compared to the low dose.

Data from the main study PB-102-F20 indicated that immunogenicity differences are observed between classical and non-classical patients, potentially related to the genotype. Results suggest that the proportion of

patients with classic phenotype experiencing IRRs may be higher than for patients with non-classic phenotype.

All patients enrolled in the clinical programme were tested for ADA at baseline and are/were retested every 6 months. Pre-existing and induced anti- pegunigalsidase alfa antibodies were observed. At baseline, 26.6 % of the patients of Cohort 4 (1 mg/kg dose EOW; switcher patients) and 33.3 % of the patients of the Cohort 2 (2 mg/kg dose E4W; switcher patients) were tested positive for IgG. Among them 96.0% and 100% respectively of these patients presented neutralising ADA. The CHMP also noted that 16.7% of the patients in naïve population had positive ADA status at baseline, suggesting that the high proportion observed in switcher patients might result from cross-reactivity.

Therefore, although data remain too limited to conclude a cross-reactivity between pegunigalsidase alfa and ADA anti-Fabrazyme, it cannot be excluded. Further investigation on cross reactivity and potential safety concern of induced hypersensitivity reactions in patients starting a treatment with pegunigalsidase alfa due to the presence of ADA previously developed against other authorised ERTs of the same indication should be followed up through routine pharmacovigilance activities. Warnings related to IRRs and hypersensitivity have been added into section 4.4 of the SmPC. Specifically, patients who have experienced severe hypersensitivity reactions with ERT infusion are identified as those in need of appropriate and readily available medical support.

Although the current data did not suggest a correlation between antibody titer and occurrence of the IRR, no firm conclusion can be drawn on frequency, severity and seriousness can be drawn due to the very limited number of cases. Altogether with the presented data on biomarkers of Fabry disease for the different ADA-subgroups, the CHMP did not support the applicant claim regarding a possible advantage of pegunigalsidase alfa on the drug immunogenicity and safety profile as compared to algasidase beta.

Regarding the development of anti-pegunigalsidase alfa antibodies, in Cohort 1 and Cohort 2, the proportion of patients treated with the 1 mg/kg dose EOW and titered with ADA is increased at Month 6 compared to baseline, and then decrease over the time. In contrast, another pattern for the patients treated with the 2 mg/kg dose E4W was observed. Regarding the every 6 months titration, the proportion of patients with ADA remains stable over the time. These findings are consistent with the fact that more frequent IRR were observed after the first year of treatment in the high dose. Although the extent of the exposure to the high dose remains limited, the presented data did not suggest a higher risk of onset of induce ADA with the 2 mg/kg E4W dose (none observed).

Furthermore, the proportion of patients with neutralising ADA among those who have ADA is rather high (overall 90% or more) suggesting a potential decrease of the efficacy, including in function of the titers. This should be further monitored through routine pharmacovigilance activities.

Finally, some patients have developed IgG anti-PEG. Four cases of serious hypersensitivity reactions were reported and likely IgE mediated (with pre-existing IgE antibodies). Since only 8 patients had pre- and post-dosing anti-drug IgE, further investigation on the relationship between IgE titer and severe hypersensitivity reactions could not be made and this should also be followed up through routine pharmacovigilance activities.

Of interest, the applicant indicated that most often IRRs occurred in patients who had already received premedication at baseline (i.e. subjects treated with ERT therapy before inclusion could be included in studies irrespective whether they had received premedication for this previous ERT). This can be understood considering that patients presenting hypersensitivity reactions to previous ERT (Fabrazyme or Replagal) may be more at risk to develop hypersensitivity reaction to pegunigalsidase alfa (see comments on cross-reactivity).

Renal events

A case of membranoproliferative glomerulonephritis led to treatment interruption and was not resolved at the time of the reporting. The Applicant indicated that a kidney biopsy had been performed which confirmed immune complex mediated membranoproliferative glomerulonephritis with subendothelial IgG deposits as well as lambda and kappa immunoglobulin deposits. The fact that immune complex deposit on kidney could lead to irreversible damages of the organs and impairment of the renal function is of concern, especially since renal impairment is one the main complications of the Fabry's disease. Additionally, the risk of local inflammatory reactions due to immune complex deposit on other organs/tissues than kidney cannot be fully ruled out. Thus in addition to the inclusion of glomerulonephritis membranoproliferative, chronic kidney disease and proteinuria as adverse drug reactions (ADRs) in section 4.8 of SmPC, a warning in section 4.4 of the SmPC was added regarding to reflect that depositions of immune complexes can potentially occur during treatment with ERTs, as a manifestation of immunological response to the product.

Other renal events reported was an acute kidney injury AE in the main study. which was considered serious, and severe, but unlikely to be related to study product, according to the applicant.

Infusion at home

Home infusion was allowed in all studies based on Investigator and the Medical Monitor' judgement. In total, almost 65% (93/142) of all patients received at least an infusion at home. In the cohort 1, it can be observed an individual mean number of 48.3 infusion, which is higher to administration on site (i.e. 36.9). Overall, the extent of the exposure at home seems sufficient to support the claim in the SmPC.

Additionally, among cases of IRR including hypersensitivity ones, all the 4 related SAE occurred during the first administration and soon after the start of the infusion (leading to immediately interrupt the treatment). Thus, no related SAE, therefore requiring prompt medical care, were observed after the first administration, and/or later in the day or week after the start of the injection. Additionally, at this stage, there is no evidence hypersensitivity reaction occurring in longer term treatment. However, data remains limited to draw strong conclusion Thus, the CHMP agreed to reflect the use of home administration in the SmPC, provided that educational materials are put in place. The important potential risk of medication error in home infusion setting will be carefully followed up through routine pharmacovigilance activities.

Due to the nature of the product, it is not expected that drug abuse, withdrawal and rebound are likely to occur, no information is available on these aspects this is considered acceptable by the CHMP. However, vertigo has been reported as common adverse reaction and thus information on influence on the ability to drive and to use machines has been reflected appropriately in the SmPC.

No safety analysis by renal impairment or hepatic impairment were performed, and no data in patients with severe renal or severe hepatic impairment are available.

2.6.10. Conclusions on the clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the SmPC. Appropriate measures including risk minimisation activities (see 2.7) have been put in place to ensure safe and effective use of the product in the recommended indication.

2.7. Risk Management Plan

2.7.1. Safety concerns

Summary of safety concerns	
Important identified risks	• Hypersensitivity Reactions (infusion related)
Important potential risks	• Medication errors in the home infusion setting
Missing information	• None

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.7.3. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Hypersensitivity Reactions (infusion related)	Routine risk minimisation measures: • SmPC section 4.4. • PL section 2 Additional risk minimisation measures: • HCP brochure • Patient/caregiver/HCP guide	Routine pharmacovigilance • Regular review of safety reports • Signal detection activities • Inclusion of discussion in the EU Periodic Safety Update Report (PSUR) Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None
Safety concern	Risk minimisation measures	Pharmacovigilance activities
Medication errors in the home infusion setting	Routine risk minimisation measures: • SmPC section 4.2 • SmPC section 6 Additional risk minimisation measures: • HCP brochure • Patient/caregiver/HCP guide	Routine pharmacovigilance • Regular review of reports • Signal detection activities • Inclusion of discussion in the EU Periodic Safety Update Report (PSUR) Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: None

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.4 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did not request alignment of the PSUR cycle with the international birth date (IBD). The new EURD list entry will therefore use the the European Birth Date (EBD) to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Elfabrio (pegunigalsidase alfa) is included in the additional monitoring list as as it is a biological product, which will be authorised after 1 January 2011.

Therefore the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Pegunigalsidase alfa is intended for long-term enzyme replacement therapy in adult patients with a confirmed diagnosis of Fabry disease, an X-linked lysosomal storage disorder caused by alterations in the gene that encodes the enzyme α-GAL-A (GLA) leading to deficient/absent enzymatic activity and resulting in glycosphingolipid accumulation with life-threatening complications.

Fabry disease is characterised by mutations in the GLA gene, resulting in decreased/undetectable levels of α-Gal-A activity in plasma or leukocytes, typically observed together with high concentrations of the substrate Gb3 and its degradation product, Lyso-Gb3 in tissues and plasma, which both correlate with organ damage.

Progressive accumulation of Gb3, Lyso-Gb3, and related lipids, leads to impaired tissue and organ function, particularly in the kidney, heart, and cerebrovascular system. In addition, involvement of the central, peripheral, and autonomic nervous systems results in episodes of pain and impaired peripheral sensation.

Fabry disease is categorised in a classic and non-classic form, based on age of symptoms onset and extent of organ involvement. Clinical onset of the classic form of disease typically occurs during childhood or adolescence and progresses to end-stage renal disease (ESRD), cardiac complications, and/or cerebrovascular disease in the fourth or fifth decade of the life. Non-classical Fabry disease, also referred to as late-onset or atypical disease, is characterized by a more variable disease course, in which patients are generally less severely affected and disease manifestations may be limited to a single organ.

Due to the X-linked nature of the disease, males are hemizygotes and females are heterozygotes and affected by the mosaicism effect.

Primary clinical manifestations of Fabry Disease in males are: neuropathic pain and acroparaesthesia; angiokeratomas; hypohidrosis; cornea verticillata; hearing loss and gastrointestinal symptoms. Renal disease is associated with progressive proteinuria accompanied by a decline in eGFR, leading over a number of years to ESRD requiring dialysis and/or kidney transplantation. Cardiac disease is associated with progressive hypertrophic cardiomyopathy with diastolic dysfunction, a variety of conduction defects and arrhythmias. Other complications are valvular disease and coronary artery stenosis of large or, more commonly, of small vessels. It has been estimated that patients with Fabry disease have a 20-fold increased risk of ischemic stroke and transient ischemic attacks compared to the general population. Both small and large vessel strokes occur, with brain regions perfused by the posterior circulation being affected more commonly than anterior circulation.

Females may express a range of clinical features including life-threatening manifestations such as cardiomyopathy, renal disease and stroke. There is considerable variation in the phenotype in heterozygous females. Age has the strongest correlation with the presence of symptoms, with the proportion of women suffering, from a particular symptom manifesting approximately a decade after that symptom is found in males.

3.1.2. Available therapies and unmet medical need

There are currently two approved classes of therapies available for patients with Fabry disease, ERT (Fabrazyme and Replagal) and pharmacological chaperone (Galafold).

Fabrazyme (agalsidase beta 1.0 mg/kg EOW) and Replagal (agalsidase alfa 0.2 mg/kg EOW) are ERTs authorised in the EU and administered by IV infusion. ERT is the longest and most successfully employed drug treatment for lysosomal storage disorders indicated across the entire spectrum of disease-causing mutations.

Both enzymes have shown effects in clinical studies with regard to the preservation of renal function [Hughes 2017; Vedder 2007; Banikazemi 2007; Eng 2001; Ortiz 2021; Wanner 2020; Germain 2007; Germain 2010; Germain 2016; Mehta 2010; Wilcox 2004; Schiffmann 2006; Schiffmann 2009]. In addition, some improvement in clearance of Gb3 from kidney cells (such as capillary endothelial cells, glomerular endothelial cells, noncapillary endothelial cells and noncapillary smooth muscle cells), and capillary endothelial cells of the myocardium and skin plasma [Eng 2001; Germain 2007; Schaefer 2009] as well as quality of life (QoL), reduction or stabilisation of cardiac mass can also be observed.

However, despite the beneficial effects described above, Fabry disease patients generally show only limited clinical improvement on the current ERTs.

Galafold (migalastat) is the only approved molecular chaperone and is only effective for a subgroup of ~30% of Fabry disease patients with amenable mutations. Galafold is approved in the United States, European Union and other countries based on reduction of renal Gb3 inclusions.

3.1.3. Main clinical studies

Studies PB-102-F01/02/03

The selected 1.0 mg/kg EOW dose was based on pharmacokinetics, pharmacodynamics/biomarker, efficacy, and safety data from studies PB-102-F01/ PB-102-F02 /PB-102-F03 which included Fabry disease patients either ERT-naïve or to having received ERT within the last 6 months (n=16). Pharmacodynamic endpoints included plasma globotriaosylsphingosine (Lyso-Gb3) concentration and renal Gb3 inclusion bodies at month 6.

Study PB-102-F20

This main phase III multicenter, randomized, double-blind study compared pegunigalsidase alfa 1 mg/kg EOW (n=52) to agalsidase beta (n=25) in adult patients with Fabry disease and impaired renal function, who were previously treated with agalsidase beta for at least one year. The study comprised a double-blind period (24 months) followed by an uncontrolled extension study. The primary objective of this study was to determine the effect of pegunigalsidase alfa on renal function as measured by annualised eGFR slope at month 12 (interim analysis), as compared to agalsidase beta.

The primary statistical objective of the interim analysis (month 12) was to test the non-inferiority (NI) of pegunigalsidase alfa versus agalsidase beta at the 5% level of significance in the ITT population. The predetermined non-inferiority margin was -3 mL/min/1.73 m^2/year.

Secondary objectives were to determine safety and effect of pegunigalsidase alfa on kidney function (eGFR as determined by serum creatinine, urine protein to creatinine ratio (UPCR)) cardiac morphology and function (MRI, stress test, electrocardiogram), biomarkers of Fabry disease (plasma lyso-Gb3 concentration, urine lyso-Gb3 concentration, plasma Gb3 concentration), severity of Fabry disease (Mainz Severity score index (MSSI)), pain (short form Brief Pain Inventory (BPI), use of pain medication), overall well-being (Quality of life questionnaire (EQ-5D-5L)), incidence of Fabry clinical events (serious renal event, cardiac event, cerebrovascular event, and non-cardiac-related death), achievement of Kidney Disease therapeutic goals.

The originally planned primary analysis in the final 2-year data set was a test for superiority over Fabrazyme to support submission of another regulatory authority. This analysis is a secondary objective of the main trial in the EU submission.

During the procedure the final CSR of study PB102-20 was submitted. In this final report, the Applicant submitted a report in which the objective of the final (24-month) analysis from superiority to non-inferiority and the primary analysis model were changed (from a random intercept random slope longitudinal mixed model comparing adjusted means of eGFR slopes to a quantile regression model comparing medians of eGFR slopes).

Study PB-102-F30

Additional data of efficacy came from a phase III multicenter, open label study in 22 adult patients with Fabry disease, who were previously treated with agalsidase alfa for at least 2 years. Patients were screened and evaluated over 3 months while continuing on agalsidase alfa and subsequently switched to receive pegunigalsidase alfa 1 mg/kg EOW over 12-months. The primary objective of this study was the assessment of safety and tolerability of pegunigalsidase alfa. The secondary objectives were: mean annualized change in estimated glomerular filtration rate (eGFR), eGFR, plasma and urine Lyso-Gb3 and plasma Gb3 concentrations, left ventricular mass index (LVMI), urine protein to creatinine ratio (UPCR), frequency of pain medication use, exercise tolerance, short form Brief Pain Inventory (BPI), Mainz Severity Score Index (MSSI), Fabry Clinical Events, and quality of life (EQ-5D-5L).

Study PB102-F50 (alternative dosing regimen)

Data on an alternative dosing regimen of 2.0 mg/kg/Q4W came from a single phase III multicenter, open label study in 29 adult patients with Fabry disease, who were previously treated with ERT (agalsidase alfa or agalsidase beta) for at least 3 years. Patients received pegunigalsidase alfa 2.0 mg/kg E4W over a 12-month period. The primary objective of this study was the assessment of safety and tolerability of this dosing of pegunigalsidase alfa. The secondary objectives were: mean annualized change in estimated glomerular filtration rate (eGFR), eGFR, plasma and urine Lyso-Gb3 and plasma Gb3 concentrations, left ventricular mass index (LVMI), urine protein to creatinine ratio (UPCR), frequency of pain medication use, exercise tolerance, short form Brief Pain Inventory (BPI), Mainz Severity Score Index (MSSI), Fabry Clinical Events, and quality of life (EQ-5D-5L).

3.2. Favourable effects

A pharmacodynamic effect of pegunigalsidase alfa was observed:

- in ERT naïve patients (PB 102-F01/02):
  - after 6 months of treatment, a decrease in renal Gb3 inclusion bodies was observed: 11 out of 14 (78.6%) subjects with available biopsies had substantial reduction of (≥50%) in their BLISS score compared to baseline and a reduction of ≥20% was achieved by 12 subjects (85.7%);
  - after 12 months of treatment, the majority (56.3%) of subjects had a greater than 50% reduction and 12.5% had a greater than 75% reduction in plasma Lyso-Gb3 levels;
- in pretreated patients (PB 102-F20/):
  - after 24 months of treatment, plasma Lyso-Gb3 values stayed stable in the study: mean (SE): +3.3 nM (1.38).

Clinical data of pegunigalsidase alfa on the renal function showed:

- in the main study (PB 102-F20 - switch from agalsidase beta) a trend of improvement in both arms compared to -8.03 mL/min/1.73 m²/year before the switch in the ITT population:
  - at month 12, the mean slopes for eGFR were -2.507 mL/min/1.73 m²/year for the pegunigalsidase alfa arm and -1.748 for the agalsidase beta arm (difference -0.749 [-3.026, 1.507];
  - at month 24, the median slopes for eGFR were - 2.514 [-3.788; - 1.240] mL/min/1.73 m²/year for the pegunigalsidase alfa arm and -2.155 [-3.805; - 0.505] for the agalsidase beta arm (difference -0.359 [-2.444 ; 1.726]);
- in a single-arm supportive study (P 102-F30 - switch from agalsidase alfa), patients tended to deteriorate less rapidly when exposed to pegunigalsidase alfa compared to their previous treatment: the mean (SE) eGFR slope of -1.19 (1.77) mL/min/1.73 m²/year at month 12 compared to -5.90 (1.34) mL/min/1.73 m²/year before switch;
- moreover, an integrated analysis of the eGFR slope (-2.80 mL/min/1.73 m²/year) up to 7.6 years of all available studies showed that the long-term effect of pegunigalsidase alfa seems to be maintained.

In further additional analyses, most patients maintained normal or near normal Left ventricular mass index (LVMI) values throughout the studies. Mean changes were small and did not suggest deterioration of LVMI

values over treatment. The severity of Fabry disease signs and symptoms according to the Mainz Severity Score Index (MSSI) and average pain severity remained stable.

3.3. Uncertainties and limitations about favourable effects

The sample size of the main study is limited (n=77). This is however expected for such a rare disease.

The main study PB-102-F20 could not be sufficiently informative on the primary endpoint/timepoint (alone), therefore other timepoints and endpoints of relevance have been considered. Since non-inferiority could not be demonstrated for the primary endpoint, all subsequently planned statistical tests for the secondary endpoints in the pre-specified hierarchy could not be carried out under adequate control of the experiment-wise type-1-error. Thus, from a methodological/statistical point of view, this study cannot provide confirmatory evidence of efficacy of pegunigalsidase alfa and moreover the data do not allow to clearly quantify the effect of pegunigalsidase alfa.

The design of this study was considered inadequate to test the non-inferiority (NI) of Elfabrio *vs*. its comparator. The prespecified NI margin (- 3 mL/min/1.73 m²/year) was based on the absolute effect of the comparator in one experience instead of retaining a modicum of its effect in comparison to no treatment and was not consistent with the expected difference in favour of pegunigalsidase (+ 1.1 mL/min/1.73 m²/year). This NI margin would imply that a loss of efficacy of pegunigalsidase alfa over the control treatment three times the expected gain of efficacy is negligible.

Furthermore the intra-patient comparisons are fraught with uncertainty due to likely regression to the mean therefore the comparison of pre- baseline and post- baseline eGFR slopes may be biased. Indeed, the reliability of the data used to evaluate the pre-baseline slopes eGFR is debatable as historical data were obtained at screening through standard local laboratory procedures, whereas creatinine values within the study were obtained using an enzymatic assay performed at a central laboratory.

3.4. Unfavourable effects

In total, 111 participants (70 males and 41 females) have been exposed to pegunigalsidase alfa (PRX-102) 1 mg/kg dose administrated every other week (EOW), of whom 83 patients were treated for at least 20 months (among them 59 for at least 30 months). Overall, the extent of the exposure is 293.3 patient-years.

TEAEs were reported in 93.7% of the subjects with a rate of 486.2 events per 100 patient-years. Related TEAEs were noted for 42.3 % of the patients with a rate of 39.6 patient-years. Additionally, 34.2 % of the patients experienced serious TEAE (19.4 per 100 patient-years), and 4.5% a related TEAE (1.7 per 100 patient-years).

The key safety point is the Infusion Related Reactions (IRRs), which are more frequent in patients with positive or induced ADA status. In the population treated with PRX-102 at the 1 mg/kg dose EOW, 37.0% (10/27) of the patients with positive ADA and 35.3% (6/17) of the patients with induced ADA experienced IRRs while the proportion was 12.7% (8/63) in patients with negative ADA. These adverse events seem overall manageable, in particular with premedication. It is noted that a substantial proportion of patients presented with positive ADA status for PRX-102 already at baseline (25.6% in patients treated with the 1 mg/kg EOW).

Among IRRs, 4 related SAEs were reported in subject treat with the 1 mg/kg dose EOW, with a rate of 1.4 events per 100 patients-year. Those were considered as IgE mediated hypersensitivity reactions: 1 case of severe bronchospasm and 3 cases of hypersensitivity, which all occurred during the first infusion of study treatment and in the two hours after start of the administration (i.e. immediate). The 4 patients recovered and were discontinued from the study.

Additionally, a related renal severe AE has been reported: a case of membranoproliferative glomerulonephritis with subendothelial IgG deposits as well as lambda and kappa immunoglobulin deposits. This AE, leading to treatment interruption, was not resolved at the time of the report.

It is also noted that the proportion of patients with neutralising ADA among those who have ADA was rather high (overall 90% or more).

3.5. Uncertainties and limitations about unfavourable effects

There are uncertainties in treatment-naive patients. Although the number of treatment-naïve subjects enrolled in the clinical program is limited, results suggest that the safety profile in IRRs might be different, with notably a higher adjusted rate of related TEAE observed in this subset compared to ERT experienced patients.

A substantial proportion of patients had positive ADA status at baseline. Considering that cross-reactivity with other ERTs is likely, there is a concern that IRRs/hypersensitivity reactions may also occur with pegunigalsidase alfa.

Regarding the risk of membranoproliferative glomerulonephritis, irreversible loss cannot be excluded. Additionally, this raises concerns regarding the potential risk of other local inflammatory reactions due to immune complex deposits on other organs/tissues than kidney, although not observed in the clinical program.

Regarding the comparative data from the study PB-102-F20 conducted versus Fabrazyme, due to the small size of the population included in study PB-102-F20, the number of TEAEs and related TEAE is low (most of the time, each related TEAE is experienced by one patient only). Thus, conclusions from the comparison of the two safety profiles is limited. Additionally, several imbalances at baseline, including in gender and history of premedication, make interpretation of the data difficult. In particular, although the current data did not suggest a correlation between antibody titer and occurrence of the IRR, no firm conclusion on frequency, severity and seriousness can be drawn due to the very limited number of cases. Altogether with the presented data on biomarkers of Fabry disease for the different ADA-subgroups, no claim regarding a possible advantage of pegunigalsidase alfa on the drug immunogenicity and safety profile as compared to algasidase beta can be made.

Finally, considering that the proportion of patients with neutralising ADA among those who have ADA is rather high (overall 90% or more), questions remain regarding on how this would impact efficacy in this population.

3.6. Effects Table

Table 78 Effects Table for Elfabrio

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Gb3 inclusions	% reduction of more than 50% reduction of GL-3 inclusion bodies per kidney IC from baseline to month 6	BLISS score	78.6% subjects with available biopsies		Descriptive statistics Data for 14 patients either ERT-naïve or to having received ERT within the last 6 months.	Study PB-102-F01
plasma Lyso-Gb3	% reduction of more than 50% and 75% change from baseline to month 12	ng/mL	56.3% of subjects had a reduction a greater than 50% 12.5% had a greater than 75% reduction in plasma Lyso-Gb3		Descriptive statistics Data for 16 patients either ERT-naïve or to having received ERT within the last 6 months.	Study PB-102-F01/02/03
eGFR Slope	Annualised change in eGFR at month 12	Mean (95% CI) mL/min/ 1.73 m^2	- 2.507 (-3.835; -1.180)	Agalsidase beta: - 1.748 (-3.585; 0.089)	Statistical non inferiority was missed for the primary endpoint. LS Mean treatment difference was -0759 (95% CI -3.026; 1.507)	Study PB-102-F20
eGFR Slope	Annualised change in eGFR at month 24	Median (95% CI) mL/min/ 1.73 m2	Month 24 (ITT population): - 2.514 (-3.788 ; - 1.240)	Agalsidase beta: Month 24 (ITT population): - 2.155 (- 3.805; - 0.505))	Non inferiority tests for the secondary endpoints could formally not be carried out under adequate control of the overall type 1-error, since statistical non inferiority was missed for the primary endpoint	Study PB-102-F20
Unfavourable Effects*						
Infusion Related Reactions	Incidence (number of events)	Events per 100 patient-years	14.0 (n=52)		More frequent in patients with positive or induced ADA status	Safety cohort 1
	Proportion of patients	%	23.4 (n=24/111)			
Serious hypersensitivity reactions	Incidence (number of events)	Events per 100 patient-years	1.4 (n=4)		IgE mediated hypersensitivity reaction	Safety cohort 1
	Proportion of patients	%	3.6 (n=4/111)			
Membranoproliferative glomerulonephritis	Incidence (number of events)	Events per 100 patient-years	0.3 (n=1)			Safety cohort 1
	Proportion of patients	%	0.9 (n=1/111)			
Neutralizing ADA	Proportion of patients at baseline	%	24.3 (n=27/111)		Among patients with positive ADA at baseline, 88.5% (23/26) have nADA	Safety cohort 1

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

It should be emphasized that pegunigalsidase alfa is a pegylated form of a human α-galactosidase-A, whose amino acid sequence is similar to the naturally occurring human enzyme, and that the efficacy of enzyme replacement therapy (ERT) with human α-galactosidase is demonstrated for the treatment of Fabry disease.

Relevant pharmacodynamic evaluations showed that pegunigalsidase alfa has a beneficial effect by decreasing both renal Gb3 inclusion bodies and plasma Lyso-Gb3 levels, which is not anticipated without effective ERT.

No final conclusion on non inferiority over agalsidase beta as measured by the annualised eGFR slope can be retrieved from the main study given that the data for the primary endpoint comparison at month 12 was not on its own sufficiently informative due to the design and size of the trial. Nevertheless, the median eGFR slopes from baseline to month 24 of pegunigalsidase and the agalsidase beta appeared close: - 2.51 versus -2.15 mL/min/1.73 m²/year, respectively.

Of note, the non-inferiority (NI) margin chosen to demonstrate the non-inferiority of pegunigalsidase alfa over agalsidase beta was inadequate as the prespecified margin was based on the absolute change of the comparator in one experience, instead of a minimum of its effect in comparison to no treatment. Moreover, comparisons of the eGRF slopes before and after study initiation may have been biased or impacted by different methods and timing of assessments, regression to the mean, or natural variability in the disease course. Finally, it should be noted that a stringent NI for eGFR would have been prohibitive in terms of sample size given the rarity of the disease and the small effect size that has actually been observed during the limited observation period in this slowly progressing disease.

Overall, the safety profile of pegunigalsidase alfa 1 mg/kg EOW is reasonably characterized and no unexpected safety concerns were observed in the intended population. The key safety point is the Infusion Related Reactions, which are importantly impacted by the ADAs' status. However, based on the current data, IRRs seem overall manageable, in particular with premedication. Nonetheless, 4 serious cases of hypersensitivity were reported, which all occurred during the first infusion of study treatment and soon after the start of the administration. Although the current data did not suggest a correlation between antibody titer and occurrence of the IRR, no firm conclusion can be drawn due to the very limited number of cases and their severity and seriousness in nature. Altogether with the presented data on biomarkers of Fabry disease for the different ADA-subgroups, no claim regarding a possible advantage of pegunigalsidase alfa on the drug immunogenicity and safety profile as compared to agalsidase beta can be made.

Additionally, a membranoproliferative glomerulonephritis due to immune complex deposits was observed, which could potentially impact the decline in renal function. This will be monitored through routine pharmacovigilance activities. Finally, uncertainties remain regarding naive patients, notably due to the very few subjects exposed.

Home infusion was allowed in all studies based on Investigator and the Medical Monitor judgement. In total, almost 65% (93/142) of all patients received at least one infusion at home not raising major safety issues.

Finally, the proportion of patients with neutralising ADA among those who have ADA is rather high (overall 90% or more), questioning how this would impact efficacy in usual practice.

3.7.2. Balance of benefits and risks

Pegunigalsidase alfa is a pegylated form of human α-galactosidase-A that is intended to supplement biological active α-GAL-A enzyme in Fabry disease patients characterised by deficient/absent enzyme activity.

The demonstration of the efficacy is based on the nature of the product (known active substance used as enzyme replacement therapy for Fabry disease), the well-understood mechanism of action, the pharmacodynamic effects observed on renal tissue that would not be expected without effective ERT and some clinical effect on the renal function.

The clinical safety profile of pegunigalsidase alfa is overall comparable to that of other authorised ERTs for Fabry disease.

Overall, the benefit/risk balance of pegunigalsidase alfa is positive in the claimed indication.

3.7.3. Additional considerations on the benefit-risk balance

The information in this section was received from the patients' organisations relating to Fabry disease; their feedback has been considered during the assessment of this procedure.

Fabry disease is a life-threatening, complex multi-organ disease. In addition to the life-threatening aspects of the disease, there are many symptoms that severely affect the patients´ wellbeing and quality of life on a daily basis (such as constant pain, GI symptoms or fatigue). There are several ERT treatments available as well as an oral medication which, however, is not available for all mutations of the disease.

Not all patients needing treatment are treated with the current treatment options. In many cases it is because the treatments are not suitable or not effective for individual patients. There are dozens of mutations in Fabry disease and it is difficult to predict which treatment will be effective for which mutation. This is why it is necessary to develop and search for new therapies that will help these individuals. There are also several unmet needs to consider in current Fabry therapies. Obviously there is the case of the Fabry brain because the current therapies don´t cross the blood-brain barrier to reach the patient´s brain. A second unmet need to consider would be the fatigue that is often combined with depression. The current therapies haven´t solved that issue completely. As far as quality of life issues are concerned, a lot of the patients find it burdensome and difficult to travel to and from hospitals every 2 weeks, so any solution to that would be more than welcome.

Female Fabry patients are often not receiving treatment because their symptoms are different from the symptoms of the male patients. They might experience less "severe" symptoms that make them not qualify for the current treatments. Their symptoms do, however, as mentioned before have serious effects on their quality of life. Many female Fabry patients experience additional problems during pregnancy and/or menopause that should also be taken into consideration in the future.

The question of acceptable side effects is complex. In patient's opinion a treatment-naive patient is ready to accept more side effects than a patient switching between products. Side effects should never be worse than the patient´s own symptoms.

3.8. Conclusions

The overall benefit/risk balance of Elfabrio is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Elfabrio is not similar to Galafold within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See Appendix on Similarity.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Elfabrio is favourable in the following indication(s):

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the marketing authorisation

- Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- Risk Management Plan (RMP)

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.
- Additional risk minimisation measures

Prior to the use of Elfabrio in each Member State in the home setting the MAH must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each Member State where Elfabrio is marketed, all Healthcare Professionals (HCP) who are expected to prescribe Elfabrio are provided with the following educational pack, which includes:

- An HCP brochure providing relevant information for the HCP to train the patient and/or caregiver to administer the product at home, which describes the following key elements:
    - ✓ checklist with eligibility criteria for home infusion
    - ✓ the need for prescribing medication to treat IRRs and that the patient/caregiver should be able to use them
    - ✓ the need for premedication if necessary (with antihistamines and/or corticosteroids) in those patients where symptomatic treatment was required.
    - ✓ the training of the person who will infuse pegunigalsidase alfa on how to identify IRRs
    - ✓ the training of the person who will infuse pegunigalsidase alfa about the preparation and administration of the product and the use of the logbook
    - ✓ the need of the logbook and its function in communication with the treating physician
    - ✓ describe the importance of the presence of a caregiver in case emergency medical care is needed
- A patient/ caregiver/ HCP guide for the administration at home which describe the following key elements:
    - ✓ Step by step instructions on the preparation and administration technique including proper aseptic technique
    - ✓ the dosing and infusion rate which will be determined by the treating physician
    - ✓ signs and symptoms of IRRs and how to treat or manage them
    - ✓ the importance of the presence of a caregiver to monitor the patient in case emergency medical care is needed
    - ✓ medication prescribed by the treating physician for IRRs or pre-medication should be available at home and should be used accordingly
    - ✓ the logbook should be used to record the infusion and any IRR, and taken to the treating physician visits

5. Appendix

5.1. CHMP AR on similarity dated 23 February 2023