Study Title

Open-Label Extension Study to Evaluate the Long-Term Safety and Efficacy of Pegunigalsidase Alfa (PRX-102) in Patients with Fabry Disease

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Confidential Declaration

CLINICAL STUDY PROTOCOL

Protocol Title: Open-Label Extension Study to Evaluate the Long-Term Safety and Efficacy of Pegunigalsidase Alfa (PRX-102) in Patients with Fabry Disease

Protocol Number:	CLI-06657AA1-04 (Previously PB-102-F60)
Investigational Product:	Pegunigalsidase alfa (PRX-102) PEGylated, chemicallymodified, recombinant human α-galactosidase A
Indication:	Pegunigalsidase alfa (PRX-102) is indicated for long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry Disease (α-galactosidase A deficiency)
Phase:	3
Protocol Version	[redacted]
Name and Address of Sponsor:	Chiesi Farmaceutici S.p.A.* Via Palermo 26/A 43122 Parma -– Italy *also reported as Chiesi throughout the text
Sponsor Medical Expert: (Clinical Research Physician)	[redacted] *Readily available in case of medical questions

GCP Statement: This study will be performed in compliance with GCP, including the archiving of essential documents

1. SYNOPSIS

TITLE: Open-label extension study to evaluate the long-term safety and efficacy of pegunigalsidase alfa (PRX-102) in patients with Fabry disease.
INVESTIGATIONAL PRODUCT: Pegunigalsidase alfa (PRX-102), a chemically modified, recombinant human α-galactosidase A.
INDICATION: Pegunigalsidase alfa (PRX-102) is indicated for long-term enzyme replacement therapy in patients with a confirmed diagnosis of Fabry disease (α-galactosidase A deficiency).
PHASE OF DEVELOPMENT: 3
INVESTIGATIONAL SITES/LOCATIONS: Multicenter.
OBJECTIVES: To evaluate the ongoing safety, tolerability, and efficacy parameters of 1 mg/kg pegunigalsidase alfa (PRX-102) every other week in adult Fabry patients who have completed studies PB-102-F20, PB-102-F30 or completed at least 48 months in PB-102-F03 study.
STUDY DESIGN: Patients will be enrolled to receive 1 mg/kg pegunigalsidase alfa (PRX-102) as intravenous infusions every two weeks (±3 days). The duration of treatment is until pegunigalsidase alfa is commercially available to the patient or at the Sponsor's discretion. The end of study will be the last patient's last visit. The last visit is defined based on the assumptions indicated above. Interim analyses may be performed for administrative purposes during the study. For the analysis, available efficacy and safety parameters will be summarized using descriptive statistics.
NUMBER OF PATIENTS (PLANNED): Up to 110 adult (male and female) Fabry patients (≥18 yrs) who completed PB-102-F20 Study, PB-102-F30 Study, or completed at least 48 months in PB-102-F03 Study.
MAIN CRITERIA FOR INCLUSION AND EXCLUSION: ***Key Inclusion Criteria:*** Eligible patients must fulfill the following inclusion criteria: 1. Completion of study PB-102-F20 or PB-102-F30 or at least 48 months in study PB-102-F03 2. The patient signs informed consent. 3. Female patients and male patients whose co-partners are of child-bearing potential agree to use a medically acceptable method of contraception. These include combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, or transdermal) supplemented with a barrier method (preferably male condom), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, or implantable) supplemented with a barrier method (preferably male condom),

intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner, or sexual abstinence.Contraception should be used for 2 weeks after treatment termination.
Key Exclusion Criteria: The following excludes a patient from study enrollment: Presence of any medical, emotional, behavioral, or psychological condition that, in the judgment of the Investigator, would interfere with patient compliance with the requirements of the study.
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION: Pegunigalsidase alfa (PRX-102) 1 mg/kg, intravenously every 2 two weeks (±3 days). Infusion and clinical observation time: All patients who completed PB-102-F20 study will be treated in PB-102-F60 study with pegunigalsidase alfa 1 mg/kg every other week. PB-102-F20 study is an ongoing double-blind study in which up to 26 patients are treated with agalsidase beta. To maintain the blinding of PB-102-F20 study, the first infusion in PB-102-F60 study of these patients will be administered intravenously over 3 hours with 2 hours of post-dosing clinical observation. Subsequent infusions will be managed according to Appendix 6, the principal investigator's (PI) evaluation. If used in PB-102-F20 infusions, premedication will be continued with the first infusion of PB-102-F60 study and then tapered down at the PI's discretion, pending the patient tolerability and according to Appendix 6. The patient will be able to return to the previously established treatment format, whether home infusion or through a predefined infusion center, once the PI agrees that it is safe to do so. Patients who completed PB-102-F30 study can continue PB-102-F60 study infusions at the same duration and setting as in PB-102-F30 study, but not less than 60 minutes, with a post-dosing clinical observation time an additional 60 minutes and the same premedication if used at PB-102-F30 study. Patients who completed at least 48 months in PB-102-F03 study can continue the PB-102-F60 study infusions at the same duration and setting as in PB-102-F03 study, but not less than 60 minutes with a post-dosing clinical observation time of an additional 60 minutes and the same premedication if used at PB-102-F03 study. Reducing the infusion time from 90 minutes to 60 minutes will be done step-wise, pending the patient's tolerability.
DURATION OF TREATMENT: Until pegunigalsidase alfa is commercially available to the patient or at the Sponsor's discretion. The end of study will be the last patient's last visit. The last visit is defined based on the assumptions indicated above.
DISCONTINUATION FROM TREATMENT:

Reasons for permanent discontinuation include the following:

- The patient experiences two or more Grade 3 toxicities or one or more Grade 4 toxicities considered by the Investigator associated with pegunigalsidase alfa's treatment.
- The patient experiences a progressive hypersensitivity or a severe hypersensitivity that is not allayed with a pre-treatment.
- The patient requests to discontinue the treatment.
- The Investigator feels that it is not in the patient's best interest to continue treatment and if the Investigator believes that the patient can no longer be compliant with the requirements of the study.
- Marketing approval is obtained, and the drug is available at the patient country.
- At the Sponsor's discretion (e.g. safety concerns, potential major protocol violation and/or fraud).

EFFICACY ENDPOINTS:

- Estimated glomerular filtration rate ($eGFR_{CKD-EPI}$).
-
- Left Ventricular Mass Index (g/m2) by magnetic resonance imaging (MRI).
- Plasma Lyso-Gb3 concentration.
- Plasma Gb3 concentration.
- Protein/Creatinine ratio, spot urine test (UPCR).
- Frequency of pain medication use.
- Exercise tolerance (Stress Test).
- Short Form Brief Pain Inventory (BPI).
- Mainz Severity Score Index (MSSI).
- Quality of life (EQ-5D-5L).
- Fabry disease Clinical events.

SAFETY ENDPOINTS:

Changes from baseline in:

- Clinical laboratory tests.
- Physical examination.
- Assessment of the injection site.
- Electrocardiography (ECG).
- Brain MRI.
- Treatment-emergent adverse events (TEAE).
- The ability to taper off infusion's pre-medication at the start of the study.
- Requirement for the use of pre-medication overall to manage infusion reactions.
- Treatment-emergent anti-pegunigalsidase alfa antibodies.

STATISTICAL ANALYSIS:

This extension study's sample size depends on the number of patients who completed PB-102-F20 study PB-102-F30 study or PB-102-F03 and enrolled into this study; up to 110 male and/or female patients.

Safety analysis will be assessed for the safety population by evaluating AEs, injection site reactions, ECG, clinical laboratory results, brain MRI, anti-pegunigalsidase alfa antibodies ability to taper off infusion pre-medication, and requirement for using premedication to manage infusions reactions.

Efficacy will be assessed for the per protocol population and summarized using descriptive statistics.

A detailed statistical analysis plan (SAP) containing the analysis information in detail including the definition of the analysis populations, derivation of variables, convention of analysis scope, and statistical methodology for the analyses of safety and efficacy parameters of pegunigalsidase alfa based on the data collected per the study protocol will be issued prior to the data base lock. In case of disagreement between the SAP and the Clinical Study Protocol, the SAP prevails

DOCUMENT APPROVAL

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements.

PRINCIPAL INVESTIGATOR

_____ _____

Signature Date

Print Name: _____

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2. LIST OF ABBREVIATIONS

AE	Adverse Event
α-GAL	α-galactosidase A
ADL	Activities of Daily Living
AKI	Acute Kidney Injury
AUC	Area under the concentration-time curve
BPI	Brief pain inventory
BLISS	Barisoni Lipid Inclusion Scoring System
CHO	Chinese hamster ovary
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
ECG	Electrocardiography
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
$eGFR_{CKD-EPI}$	Estimated glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration
ERT	Enzyme replacement therapy
ESRD	End-stage renal disease
FOS	Fabry Outcome Survey
Gb3	Globotriaosylceramide
GCP	Good Clinical Practice
GSA	Gastrointestinal Symptoms Assessment
IC	Informed consent
IDP	Investigational Drug Product
IEC	Institutional ethics committee
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IR	Infusion rate
IRB	Institutional Review Board
IRR	Infusion-related reaction
ITT	Intent-to-Treat

AE	Adverse Event
LOCF	Last observation carried forward
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMI	Left ventricular mass index
Lyso-Gb3	Globotriaosylsphingosine
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MSSI	Mainz Severity Score Index
PEG	Polyethylene glycol
PI	Primary investigator
PP	Per Protocol
PT	Prothrombin time
PTT	Partial thromboplastin time
SAE	Serious adverse event
TIA	Transient ischemic attack
t 1/**2**	Half life

3. ETHICAL CONDUCT OF THE STUDY AND REGULATORY REQUIREMENTS

3.1 Institutional Review Board

An Institutional Review Board (IRB) or Institutional Ethics Committee (IEC) will review the study protocol and any amendments. The IRB or IEC will also review the informed consent forms, their updates (if any), and any written materials given to the patients. A list of all IRBs and IECs and contact information will be included in the study report.

3.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, in compliance with the approved protocol, GCP, and applicable regulatory requirements.

3.3 Patient Information and Consent

The investigator will obtain a freely given written consent from each patient after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other study aspects relevant to the patient's decision to participate. The consent forms must be signed and dated by the patient before he/she is exposed to any protocol-specific procedure.

The investigator will explain that the patients are entirely free to refuse to enter the study or to withdraw from it at any time, without any consequences for their further care, and without the need to justify their decision.

The patient will receive a copy of the patient's information and the signed informed consent forms.

The patient will be informed if information becomes available that may be relevant to his/her willingness to continue participation in the study.

Each patient will be informed that a monitor or a health authority inspector may review the portions of their source records and source data related to the study in accordance with applicable regulatory requirements. Data protection and confidentiality will be handled in compliance with local laws.

4. INTRODUCTION

Fabry disease is a progressive lysosomal storage disease that is seriously debilitating and ultimately life-threatening. It is caused by an X-linked deficiency of the enzyme α-galactosidase A (α-GAL) and affects both males and females. The disease is characterized by the subnormal or absent activity of α-GAL. Clinical onset of the disease typically occurs during childhood or adolescence (Schaefer et al., 2009 [27]) and will progress to end-stage renal disease (ESRD), cardiac complications, and cerebrovascular problems in the fourth or fifth decade of life (Wilcox et al., 2008 [40]). Although Fabry disease is an X-linked disorder, females are also affected and develop manifestations of the disease due to lack of cross-correction between cells with normal α-GAL activity (mutated X chromosome is inactivated) and cells with enzyme deficiency (non-mutated X chromosome is inactivated). The clinical abnormalities in females are more variable, and of later onset compared to males (Schiffmann, 2009 [31])

Fabry disease is regarded as a rare disease, and it is estimated that 1 in 40,000 males has the disease, whereas the estimated prevalence in the general population is 1 in 117,000 (Meikle et al., 1999 [22]). Random screening of 37,104 newborns for Fabry disease in Italy indicated the prevalence in males to be 1 in 3,100 (Spada et al., 2006 [33]). The large difference in statistics was explained as being due to the inclusion of often undiagnosed patients with the non-classic form of Fabry disease (late-onset), with the ratio of late-onset to classical being 11 to 1 (Spada et al., 2006). Random screening of 110,027 newborns in Taiwan discovered the incidence in males to be about 1 in 1,500 (Lin et al., 2009 [20]). This study determined there were still significantly fewer females affected than males, with approximately 1 in 17,000 affected; this prevalence is still a much greater incidence than was previously thought for males (Lin et al., 2009). While lysosomal storage diseases are individually rare, the cumulative incidence of known patients and the undetected reservoir of undiagnosed patients with these diseases constitute a significant total incidence of disease with a large societal impact.

α-GAL is a lysosomal enzyme that primarily catalyzes the hydrolysis of the glycolipid globotriaosylceramide (Gb3) to galactose and lactosylceramide. Fabry disease is characterized by massive storage of Gb3, predominantly in the endothelial cells of the vascular system, cardiomyocytes, neuronal cells, and kidney podocytes. Progressive accumulation of Gb3, and related lipids, leads to impaired tissue and organ function. The ultimate consequence of glycolipid deposition in the vasculature and other tissues is an end-organ failure, particularly in the kidney, but also the heart and cerebrovascular system (Schiffmann, 2009 [31]). This organ failure is brought on by Gb3 and globotriaosylsphingosine (Lyso-Gb3) activation of the innate immune system via the toll-like receptors on the dendritic cells. In the kidney, Gb3 accumulation is associated with an increase of transforming growth factor-beta and the dedifferentiation of the endothelial cells, leading to renal fibrosis. Fibrosis is also a typical feature of heart involvement in Fabry disease. Endomyocardial biopsies show infiltration of lymphocytes and macrophages, suggesting a role for inflammation in causing tissue damage (Rozenfeld and Feriozzi, 2017 [26]). In addition, involvement of the central, peripheral, and autonomic nervous systems results in episodes of pain and impaired peripheral sensation. Vascular changes in the skin also result in angiokeratomas (Hoffmann et al., [14] 2009). The mechanism by which α-GAL deficiency and glycolipid accumulation cause such a wide variety of complications are not well understood. Based on the

pathology of Fabry disease, the ongoing accumulation of alpha-*D*-galactosyl moieties, particularly of Gb3, appears to be a chronic toxicity state (Schiffmann, 2009 [31]). Aerts et al., 2008 [01], reported that globotriaosylsphingosine (Lyso-Gb3), a Gb3 metabolite, is dramatically increased in the plasma of male Fabry patients and plasma and tissues of Fabry mice and may have an important role in the pathogenesis of Fabry disease. Increased levels of Lyso-Gb3 also occur in symptomatic Fabry females (Van Breemen et al., 2011 [35]).

As Fabry disease is an X-linked disorder, the prevalence of the mutation is predicted to be two times higher in women than in men. There is considerable variation in phenotype in heterozygous females. However, despite the X-linked nature of the disease, heterozygous and therefore tissue-mosaic females can be as severely affected by Fabry disease as hemizygous males, experiencing progressive, multi-organ involvement, reduced quality of life, and reduced life expectancy. Case-finding studies have reported mutations that are known to be associated with Fabry disease in 0.3-2.4% of women who had an unexplained stroke, hypertrophic cardiomyopathy, or renal failure requiring hemodialysis. A study comparing men and women with Fabry disease, using data from the Fabry Outcome Survey (FOS), showed no significant differences between men and women for most clinical features evaluated after four years of treatment. Overall, both sexes responded to enzyme replacement treatment in a similar way (Hughes et al., 2011 [17]).

Enzyme replacement therapy (ERT), by exogenous administration of purified recombinant enzyme, is nowadays among the most successfully employed drug treatments for lysosomal storage disorders. The first disorder for which this treatment modality has proven effective is type 1 Gaucher disease (Barton et al., 1991 [02]; Hollak et al., 1995 [15]). This success has paved the way for ERT development for other lysosomal storage disorders, including Fabry disease.

Recombinant human α-GAL metabolizes the accumulated Gb3 in patients. Currently, two ERTs using this enzyme are commercially available; agalsidase-alpha (Replagal®), approved in Europe, and agalsidase-beta (Fabrazyme®), which was approved in Europe and the United States. Both recombinant enzymes are comparable in their properties and differ only slightly in glycan composition (Blom et al., 2003 [05]). They are produced using different protein expression systems and are administered at different doses. Fabrazyme® is produced in Chinese Hamster Ovary (CHO) cells and is administered by IV infusion every two weeks at a dose of 1 mg/kg. Replagal® is produced in a human cell line (stably expressed in human foreskin fibroblast) (Schiffmann et al., 2000 [28]) and is administrated by IV infusion every two weeks at a dose of 0.2 mg/kg. Both products have shown their efficacy in clinical studies with regard to clearance of Gb3 from plasma, kidney cells (such as capillary endothelial cells, glomerular endothelial cells, noncapillary endothelial cells, and noncapillary smooth muscle cells), and capillary endothelial cells of the cardiac and skin (Eng and Guffon et al., 2001 [10]; Germain et al., 2007 [13]; Schaefer et al., 2009 [27]; Deegan, 2012 [07]). In addition, ERT with both products leads to improvement in quality of life evaluated by SF-36 questionnaire, reduction or stabilization of cardiac mass, preservation of renal function, and slowing down the decline of glomerular function (Wilcox et al., 2004 [39]; Schiffmann et al., 2006 [29]; Germain et al., 2007; Schiffmann 2009 [31]). The improvements observed in the quality of life were scored and found to be significant in physical functioning, role emotional, body pain, and standardized physical component scale (Germain et

al., 2007). Although these findings are encouraging, the clinical effects of the current treatment of Fabry patients are not as robust as anticipated and show only limited clinical improvement (Schaefer et al., 2009; Lidove et al., 2010 [19]; El Dib et al., 2011 [08]).

One of the major factors responsible for the limited efficacy of current treatment is the presence of irreversible organ damage. Another factor that may contribute to this is the characteristics of the current ERTs (such as short pharmacokinetic half-life and dose regimens), which seem to be insufficient in preventing the chronic toxic effect of Gb3. One way to improve enzyme bioavailability may be to extend circulation residence and tissue half-life. A third factor, which may influence treatment outcome, is the induction of antibodies towards the recombinant proteins (Hollak et al., 2009 [16]). The emergence of antibodies with *in vivo* neutralizing capacity is frequently encountered in treated Fabry disease patients, resulting in inhibition of enzyme activity and adversely affecting Gb3 clearance (Hollak et al., 2009 [16]). In early clinical studies, 53 to 88% (Schiffmann et al., 2006 [31]; Eng and Banikazemi et al., 2001 [09]; Eng and Guffon et al., 2001 [10]) of male patients developed these immunoglobulin G (IgG) antibodies within the first six months of treatment. Regarding treatment outcome, it was shown that antibodies against α-GAL interfere with the clearance of Gb3 from plasma, urine (Linthorst et al., 2004 [21]; Vedder et al., 2008 [36]), and from the tissue (Benichou et al., 2009 [03]). The cross-reactivity of α-GAL antibodies suggests that it is unlikely that switching from one recombinant protein to the other may prevent the immune response and related effects (Linthorst et al., 2004; Hollak et al., 2009). Currently, administering a higher dose of the recombinant enzyme is an effective way to overcome the increase in Gb3 observed in patients' urine associated with neutralizing antibodies. However, this approach is not considered a long-term solution (Vedder et al., 2008 [36]; Hollak et al., 2009 [16]). A later study has shown that IgG levels are dose-dependent (Smid et al., 2013 [32]).

Protalix has developed pegunigalsidase alfa (PRX-102), a chemically modified (polyethylene glycol [PEG] addition) recombinant human α-GAL, with amino acid additions on both the N and C-terminus, expressed in a tobacco cell culture which results in oligomannose glycosylation. As a result of these modifications, pegunigalsidase alfa exhibits more stabilized enzymatic activity, extended circulation residence time, and enhanced bioavailability of the enzyme relative to the commercial drug. Therefore, pegunigalsidase alfa provides the continuous presence of enzyme over the 2-week dosing interval (Kizhner et al., 2015 [18]).

Studies have shown that human α-GAL is a non-covalently bound homodimeric glycoprotein (Garman et al., 2004 [11]) and that dimerization is important for the enzymatic activity and stability of the enzyme (Bishop et al., 1988 [04]). The chemical modification of pegunigalsidase utilizes the reagent bis-NHS-PEG and renders PEGylated protein subunits, the majority of which are crosslinked into homodimers. In addition, the PEGylation modification may have an additive value through increasing drug retention time in blood and bioavailability (Veronese et al., 2005 [37]; Veronese et al., 2008 [38]). Preliminary non-clinical data show that this modification improves pegunigalsidase alfa stability *in vitro* under lysosomal and plasma conditions and extends circulation residence and bioavailability *in vivo*, probably due to stabilization of its quaternary structure. Therefore, the modifications in pegunigalsidase alfa have the potential to improve the efficacy of ERT.

Pegunigalsidase alfa enhanced pharmacokinetic properties including a half-life ($t_{½}$) of approximately 80 hours and AUC (Area under the concentration-time curve) of approximately 400,000 ng*hour/mL for the 1 mg/kg dose after one day of treatment (Day 1), which are believed to be the result of the covalent cross-linking to make pegunigalsidase alfa a more stable homodimer. This extended exposure of the target organs to enzyme may be important for improving efficacy similar to what Schiffmann and colleagues (Schiffman, R 2007 [30]) showed with weekly compared to every other week agalsidase alfa (Replagal®), which reports, in comparison, elimination $t_{½}$ of 108±17 minutes and 89±28 minutes for males and females, respectively (Replagal® EMA, 2006 [24]). Agalsidase beta (Fabrazyme®) reports a comparable terminal $t_{½}$ within the range of 45-102 minutes, independent of concentration (Genzyme FDA, 2003 [12]).

In view of the data to date, it is concluded that dosing of pegunigalsidase alfa at 1 mg/kg every two weeks offers an appropriate treatment regimen for attenuating disease progression.

Risk/benefit assessment

As of 12 October 2020, there were 224 patient-years of exposure for the regimen of 1.0 mg/kg PRX-102 administered biweekly and 67 patient-years for the regimen of 2 mg/kg administered every 4 weeks, which is considered sufficient for the assessment of safety and tolerability in a rare disease. Both treatment-naive and ERT-experienced patients have been included in clinical studies. Benefits have been seen in reduction in the tissue Gb3 accumulation (evaluated on kidney biopsies using the Barisoni Lipid Inclusion Scoring System – BLISS), in the biomarker of plasma lyso-Gb3, stability or improvement in kidney and cardiac function, and stabilization on other indicators of clinical progression such as scores on the Mainz Severity Score Index, the Brief Pain Inventory, and quality of life questionnaires, thereby demonstrating achievement of the therapeutic goals of Fabry disease treatment. Given the progressive nature of the disease, stabilization of overall clinical progression can be considered a therapeutic success.

Currently available safety data indicate that the drug has a favorable safety profile and is well tolerated. There have been no deaths related to treatment; few serious adverse events related to study product, infusion-related reactions (IRRs) in particular. In general, IRRs are well-controlled through pausing and re-starting the infusion and/or through the use of premedication (both before and during the infusion) and/or through slowing down the infusion rate. IRRs recorded in the trials were for the majority of the cases mild to moderate, however the very few events that led to study discontinuation were severe IRRs and occurred at the beginning of the treatment. All patients were appropriately treated and quickly fully recovered. Discontinuations from the study have been related to hypersensitivity reactions (1 event of bronchospasm in F01, 2 events of type I hypersensitivity in F30, 1 event of hypersensitivity in F20, 1 event of type I hypersensitivity in the Early Access Program) and consent withdrawal by the study participants, for various reasons (not safety related). In addition, there is a low rate of treatment-induced antibody formation, which is important as the need for ERT treatment is lifelong. Treatment with the posology at 2mg/kg every 4 weeks was equally safe, with very few SAEs reported in the F50, none related, and no serious or

severe IRRs. Overall, the risk/benefit profile is positive, and it can be concluded that a regimen of PRX-102 at 1.0 mg/kg every 2 weeks, and the alternative posology at 2 mg/kg every 4 weeks, offer an appropriate treatment for attenuating the progression of Fabry disease. Full details on the properties of pegunigalsidase alfa and data related to its nonclinical and clinical development are provided in the Investigator's Brochure.

5. STUDY OBJECTIVES

To evaluate the ongoing safety, tolerability, and efficacy parameters of pegunigalsidase alfa in adult Fabry patients who have completed studies PB-102-F20 and PB-102-F30 studies or at least completed 48 months on PB-102-F03 study.

6. INVESTIGATIONAL PLAN

6.1 Overall Study Design and Plan – Description

This will be an open-label, multicenter study of 1 mg/kg of pegunigalsidase alfa intravenous infusion every two weeks (±3 days) to evaluate the safety, tolerability, and efficacy of pegunigalsidase alfa in adult Fabry patients (≥18 years of age).

The duration of treatment will be until pegunigalsidase alfa is commercially available to the patient or at the Sponsor's discretion. The end of study will be the last patient's last visit. The last visit is defined based on the assumptions indicated above.

Disease parameters evaluated during PB-102-F20, PB-102-F30, and PB-102-F03 studies will continue to be assessed in this extension protocol (CLI-06657AA1-04, previously PB-102-F60 study).

6.2 Discussion of Study Design

The extension study may provide additional, long-term information on tolerability, safety, and efficacy in patients treated with pegunigalsidase alfa.

6.3 Selection of Study Population

6.3.1 Inclusion Criteria

Eligible patients must fulfill the following inclusion criteria:

1. Completion of PB-102-F20 or PB-102-F30 studies, or at least 48 months in PB-102-F03 study.

2. The patient signs informed consent.

3. Female patients and male patients whose co-partners are of child-bearing potential agree to use a medically acceptable method of contraception. These include combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of

ovulation (oral, intravaginal, or transdermal) supplemented with a barrier method (preferably male condom), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, or implantable) supplemented with a barrier method (preferably male condom), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner, or sexual abstinence.. Contraception should be used for 2 weeks after treatment termination.

6.3.2 Exclusion Criteria

Presence of any medical, emotional, behavioral, or psychological condition that, in the judgment of the Investigator, would interfere with the patient's compliance with the requirements of the study.

6.3.3 Removal of Patients from Therapy or Assessment

Reasons for permanent discontinuation include the following:

- The patient experiences two or more Grade 3 toxicities or one or more Grade 4 toxicity considered by the investigator to be associated with pegunigalsidase alfa treatment (CTCAE v4.03, 2010 [34]).
- The patient experiences a progressive hypersensitivity or a severe hypersensitivity that is not allayed with pre-treatment.
- The patient requests to discontinue the treatment.
- The Investigator feels that it is not in the patient's best interest to continue the treatment and/or if the Investigator believes that the patient can no longer be compliant with the requirements of the study.
- Marketing approval is obtained, and the drug is available at the patient country.
- At the Sponsor’s discretion (e.g. safety concerns, potential major protocol violation and/or fraud).

For any discontinuation, the Investigator will obtain all the required details and document the date and the main reason for the premature termination. If the reason for discontinuation is an AE, the specific event or the main laboratory abnormality will be recorded in the eCRF. The Investigator will make thorough efforts to document the outcome. The Investigator will attempt to continue to follow the patient for the full duration of the study or at least for 60 days following discontinuation. If circumstances prevent the patient from completing all visits, every attempt will be made to complete all procedures listed in Section 9 for the last visit.

7. STUDY PRODUCT

7.1 Study Medication Supply

The study drug will be supplied to the clinical site under the responsibility of the Sponsor, who will also provide the Pharmacist/Investigator with appropriate certificates of analytical conformity, if required.

7.2 Description and Formulation of Study Product

Pegunigalsidase alfa is a purified recombinant, plant cell-expressed chemically modified human α-GAL, described in detail in the Investigator's Brochure.

Each vial contains 10 mL (2.0 mg/mL) of the following contents in liquid form:

- 20 mg pegunigalsidase alfa.
- [redacted] Sodium chloride (NaCl).
- [redacted] mM Sodium citrate ([redacted]

7.3 Study Drug Dosage and Preparation

Pegunigalsidase alfa 2.0 mg/mL vials are stored at 2-8°C (36-46°F).

The individual dose for each patient (1 mg/kg) will be prepared according to patient weight at the screening visit (the last visit of the previous study). The dosage will be adjusted to patient weight obtained at Visits Screening, 14, 27, 40, 53, 66, 79, 92, 105,118, 131, 144, 157, 170, 183, 196 and 209 if the weight changes by 25% from the previous adjustment.

The total volume of the infusion will be adjusted with normal saline (0.9% NaCl) to a final volume of:

- 150 mL/infusion for patients weighing up to 70 kg.
- 250 mL/infusion for patients weighing between 70 kg-100 kg.
- 500 ml/infusion for patients weighing above 100 kg.

The infusion volume may be re-calculated only if dosage adjustment was performed.

7.4 Description of Comparator Product

Not applicable.

7.5 Study Drug Administration

All patients will receive 1 mg/kg pegunigalsidase alfa, administered intravenously every two weeks (±3 days).

All patients who completed PB-102-F20 study will be treated in PB-102-F60 study with 1 mg/kg pegunigalsidase alfa every other week. PB-102-F20 study is a double-blind study in which patients have been enrolled after treatment with agalsidase beta and then randomized (2:1) to

pegunigalsidase alfa or continue on agalsidase beta. In order not to compromise the blind of PB-102-F20 study, the first infusion of pegunigalsidase alfa in these patients in the PB-102-F60 study will be administered intravenously over 3 hours with 2 hours of post dosing clinical observation. The infusion will be managed from the second infusion and onwards according to Appendix 6, Investigator's evaluation; Sponsor's Medical Expert will be notified and available for consultation if needed. With the first infusions, premedication, if used as part of PB-102-F20 infusions, will be continued and then tapered down at the Investigator's discretion pending the patient's tolerability and according to Appendix 6. The patient will return to the previous treatment set up, either home infusion or predefined infusion center, once the Investigator agrees that it is safe to do so; Sponsor's Medical Expert will be notified and available for consultation if needed.

Patients who completed the PB-102-F30 study or PB-102-F03 study can continue the infusions at the same duration achieved in the previous study but not less than 60 minutes, with a post-dosing observation time of an additional 60 minutes and the same premedication, if used.

The reduction of the infusion time from 90 minutes to 60 minutes will be conducted in a step-wise manner pending the patient's tolerability and per Investigator's discretion; Sponsor's Medical Expert will be notified and available for consultation if needed.

7.6 Packaging and Labeling

The drug product is packed in vials containing 20 mg pegunigalsidase alfa (2.0 mg/mL), [redacted] NaCl, and [redacted] mM sodium citrate [redacted]

The study drug is packed in 15 mL clear injection glass vials. Rubber stoppers used for closure and are sealed with aluminum seals.

The study drug will be supplied to the Site Pharmacies and Central Pharmacies of the Homecare Service Providers in the form of treatment kits. Each treatment kit is composed of a labeled box containing 15 labeled vials.

7.7 Conditions for Storage and Use

The study product is stored at 2-8°C (36-46°F).

7.8 Method of Assigning Patients to Treatment Groups

The dose of all patients will be 1 mg/kg, thus one treatment group.

The patient number will continue to be as the one assigned in PB-102-F20, PB-102-F30 or PB-102-F03 studies.

7.9 Dispensing, Compliance, and Accountability

The Investigator or the designated/authorized representative, is responsible for the management of all the study medications to be used for the study. Study drugs should be stored in a locked and secure storage facility with access limited to those individuals authorized to dispense the study medication.

An inventory will be maintained by the Investigator or pharmacist (or other designated individual) to include a signed account of all the study drugs received, dispensed and returned during the trial. The Clinical Service Provider, Clinical Trial Supply Vendor and/or Homecare Service Provider(s) will provide drug accountability forms to assist the pharmacist in maintaining current and accurate inventory records covering receipt, dispensing, and the return of investigational drug supplies. When a shipment is received, the pharmacist and/or Investigator will verify the quantities received and return the acknowledgment to the Clinical Trial Supply Vendor. If a temperature excuriosn outside of the permitted ranges occurs, the drug will not be used without Sponsor's approval in writing. The pharmacist investigational drug accountability record includes the patient number to whom the drug is dispensed, the quantity and the date of dispensing, and any returned or unused drug, as well as a complete record of IDP storage temperature. This record is used in addition to any drug accountability information recorded on the Case Report Form (CRF). These records will be readily available for inspection by a monitor and/or Chiesi audits and are open to regulatory authority inspection at any time.

At the conclusion or termination of the study, the Investigator or the pharmacist must conduct and document a final inventory of all used and unused study drug. An explanation will be given for any discrepancies.

All study drugs supplied, used or unused, will be returned to Chiesi or to the designated Clinical Service Provider/Clinical Trial Supply Vendor under the Sponsor's responsibility or destroyed directly at the Site. In this case, a destruction certificate must be provided by the Site and filed both by the Site and by the Sponsor. Destruction may not occur unless previously authorized in writing by Chiesi Farmaceutici S.p.A.

7.10 Prior and Concomitant Therapy

Medications having the potential to interfere with the evaluation of efficacy are excluded throughout the trial. The following medications are strictly prohibited during the study:

- Fabrazyme® (agalsidase beta).
- Replagal® (agalsidase alfa).
- Galafold™ (migalastat).
- Any other investigational drug for treating Fabry disease.

8. EFFICACY AND SAFETY ASSESSMENTS

8.1 Efficacy Variables

The following efficacy endpoints evaluated in PB-102-F03, PB-102-F20 and PB-102-F30 studies will continue to be measured in this extension study. Baseline values for this extension study will be the values from the last infusion of PB-102-F03, PB-102-F20, or PB-102-F30 studies. As one out of every three patients in the PB-102-F20 Study will be transitioning from agalsidase beta to

pegunigalsidase alfa, patients will undergo more frequent monitoring during the first year in CLI-06657AA1-04 (previously PB-102-F60).

- The mean change of the estimated glomerular filtration rate ($eGFR_{CKD-EPI}$) calculated from the serum creatinine values, will be evaluated every six months, up to the end of the study. For patients who completed the PB-102-F20 study, serum creatinine will be measured at more frequent times (Visits 3, 7, 10, and 20) in the first year of the Study (Study Flow Chart).
- Plasma Lyso-Gb3 and Plasma Gb3 concentrations will be evaluated every six months up to the end of the study. For patients who completed PB-102-F20, plasma Lyso-Gb3 and Plasma Gb3 concentration will also be measured every three months for the first year of the study.
- Urine protein/Creatinine ratio (UPCR), measured by a spot urine sample, every six months up to the end of the study. For patients who completed PB-102-F20, UPCR will also be measured at Visit 7.
- Short Form Brief Pain Inventory (BPI) will be evaluated every six months up to the end of the study. (See Appendix 5. Brief Pain Inventory- BPI (Short Form)).
- Left ventricular mass (LVM) myocardial fibrosis and ejection fraction (EF) assessed by cardiac magnetic resonance imaging (MRI) - every 12 months up to the end of the study. For patients for whom cardiac MRI cannot be performed, LVM and EF will be assessed by echocardiography every 12 months up to the end of the study.
- Stress Test will be performed every 12 months up to the end of the study.
- Mainz Severity Score Index (MSSI) will be calculated every 12 months up to the end of the study.
- The frequency of pain medication and premedication use will be evaluated throughout the study (all Visits).
- Quality of life (Appendix 7, EQ-5D-5L) evaluation will be performed every six months up to the end of the study.

8.2 Safety Variables

Safety will be assessed by the frequency, severity, and duration of treatment-emergent adverse events (TEAE). including clinically significant laboratory abnormalities, ECG changes from baseline test, brain MRI changes from baseline, physical examination findings, assessment of the injection site reactions, and the existence of anti-pegunigalsidase antibodies after administration of the study drug.

In addition, the following parameters will be measured:

- Ability to taper off infusion premedication at the start of the study.

- The requirement for the use of premedication to manage infusion reactions.

8.2.1 Clinical Laboratory

- Hematology: total white blood cell count, hemoglobin, and platelets.

- Biochemistry: sodium, potassium, glucose, blood urea nitrogen, calcium, phosphate (inorganic), uric acid, total protein, albumin, bilirubin (total), alkaline phosphatase, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, lactate dehydrogenase, creatine Phosphokinase (CPK).

- Serum Enzymatic Creatinine and Cystatin C. In case a patient reaches an end-stage renal disease condition, the Principal Investigator and the Sponsor's Medical Expert will discuss whether or not to continue collecting the Enzymatic Creatinine and Cystatin C.

- Urinalysis: dipstick for the presence of blood, glucose, ketones, Nitrite, Leukocyte, and protein.

8.2.2 Anti-Pegunigalsidase alfa Antibodies

Anti-pegunigalsidase antibodies, including neutralizing antibodies (in patients having a positive IgG antibody response), will be assessed using validated bioanalytical methods.

- Anti-pegunigalsidase antibodies will be assessed every six months up to the end of the study. For patients who completed the PB-102-F20 study, anti-pegunigalsidase antibodies will be measured at more frequent times (Visits 3, 7, 10, and 20) in the first year of the study (see Study Flow Chart).

Anti-Drug Immunoglobulin E (IgE) antibodies will be assessed in any event of a hypersensitivity reaction, following the Sponsor's request.

8.2.3 Adverse Events

8.2.3.1 Adverse Events and Serious Adverse Events

An Adverse Event (AE) is any untoward medical occurrence in a patient participating in a clinical trial. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of the study medication, whether or not considered related to the study medication. AEs will be collected from the start of treatment until 90 days following the final visit dose.

This definition also includes accidental injuries, reasons for any change in medication (drug and dose) other than planned titration, reasons for admission to a hospital, or reasons for surgical procedures (unless for minor elective surgery for a pre-existing condition). It also includes AEs commonly observed, and AEs anticipated based on the pharmacological effect of the study

medication. Any laboratory abnormality assessed as clinically significant by the Investigator must be recorded as an AE.

A Treatment-Emergent Adverse Event (TEAE) is any AE occurring after the start of study medication and within the time of residual drug effect (20 days after the last administration of the study medication), or a pre-treatment AE or pre-existing medical condition that worsens in intensity after the start of study medication and within the time of residual drug effect.

AEs should be recorded as diagnoses, if available. If not, separate sign(s) and symptom(s) are recorded. One diagnosis/symptom should be entered per record. Treatment-related hypersensitivity/infusion reactions are defined in Appendix 2 and can be defined as a single AE at the Investigator's discretion.

Note that death is not an event, but the cause of death is. An exception is the event of sudden death of an unknown cause. Note that hospitalization is not an event; however, the reason for hospitalization is. Procedures are not events; the reasons for conducting the procedures are. In general, only the reason for conducting the procedure will be captured as an AE. However, if deemed necessary by the Investigator, a procedure can be captured along with the reason for conducting the procedure.

An overdose or medication error is not an AE unless it is temporally associated with an unfavorable or unintended sign or symptom.

Each AE is to be classified by the investigator as serious or non-serious. A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that occurs at any dose:

- Results in death.
- Is life-threatening (i.e., an immediate risk of death).
- Requires in-patient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is associated with a congenital anomaly/birth defect.
- Is an important medical event.

An AE caused by an overdose or medication error is considered serious if a criterion listed in the definition above is fulfilled.

Important AEs that may not result in death may not be life-threatening or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient's safety or may require medical or surgical intervention to prevent one of the outcomes listed above.

SAEs also include any other event that the investigator or sponsor judges to be serious or defined as serious by the regulatory agency.

The investigator will report all directly observed AEs and all AEs spontaneously reported by the trial patient using concise medical terminology. In addition, each trial patient will be questioned about AEs. The question asked will be, "Since you began taking the study medication, have you had any health problems?"

An Infusion Related Reaction is a reaction to the infusion of pharmacological or biological substances. Symptoms may appear within minutes to 2 hours following the end of the infusion and may include pruritus, flushing, swelling, dyspnea, bronchospasm, and hypotension. IRR's may be evaluated up to 24 hours from occurrence.

8.2.3.2 Procedures for Assessing, Recording, and Reporting AEs and SAEs

Throughout the study, the Investigator will closely monitor each patient for evidence of drug intolerance and the development of clinical or laboratory evidence of AEs. All AEs (expected or unexpected) that occur during the study, whether observed by the Investigator or by the patient, and whether or not thought to be drug-related, will be reported and followed until resolution or until they become stable.

The description of the AE will include a description of the event, start date, stop date, intensity, whether it was serious or not, relationship to test drug, any change in the study drug dosage, if it was fatal, and if treatment was required.

Events will be coded to one of the following intensity categories below based on the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03; June 14, 2010:

Severity	Definition
Mild (Grade 1)	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Moderate (Grade 2)	Minimal, local, or noninvasive intervention indicated, limiting age-appropriate instrumental ADL*
Severe (Grade 3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**
Very Severe (Grade 4)	Life-threatening consequences: urgent intervention indicated.

*Activities of Daily Living (ADL) - Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refers to bathing, dressing, and undressing, using the toilet, taking medications, and not bedridden.

Events will be coded into one of the following causality categories as defined below:

Category	Definition

Unrelated	Clearly and incontrovertibly due only to extraneous causes and does not meet criteria listed under possible or probable.
Unlikely	Does not follow a reasonable temporal sequence from the administration. It May have been produced by the patient's clinical state or by environmental factors or other therapies administered.
Possible	Follows a reasonable temporal sequence from administration but may have also been produced by the patient's clinical state, environmental factors, or other therapies administered.
Probable	Clear-cut temporal association with administration with improvement on cessation of investigational medicinal product or reduction in dose. Reappears upon rechallenge. Follows a known pattern of response to the investigational medicinal product.
Definitely	There is evidence of exposure to the test product, for example, reliable history or acceptable compliance assessment; the temporal sequence of the AE onset relative to the drug is reasonable; the AE is most likely to be explained by the drug treatment than by another cause; the challenge is positive; re-challenge (if feasible) is positive; the AE shows a pattern consistent with previous knowledge of the drug treatment.

AEs with the causality assessed as unrelated or unlikely are categorized as not related to study medication.

AEs with the causality assessed as possible, probable, or definitely are categorized as related to study medication and adverse drug reactions.

All SAEs must be reported immediately (no more than 24 hours after becoming aware of the event) by entering the information about the event in the eCRF forms. The [redacted] safety contact will be notified of the event by the eCRF system. The information must be provided by the completion of the SAE investigator form. At a later date, the [redacted] Safety Contact will report all information to Chiesi Global Pharmacovigilance, the Clinical Project Manager and the Sponsor Medical Expert. In case the eCRF system is not available, the Investigator must immediately contact the assigned CRO safety contact (no later than 24 hours of becoming aware of the event). A paper back-up SAE form will be distributed to the sites, and will be used in case of eCRF unavailability.

Reporting of SAEs from the Treating Physician is from the time of patient's informed consent signature and until the patient's treatment participation ends. After this date, even if no active monitoring of patient is required, SAEs occurring to a patient should be reported if the Treating Physician becomes aware of them.

Up to the closure of the site, SAE reports should be reported to [redacted] Safety Contact. New serious adverse events occurring after the site is closed should be reported directly to Chiesi Safety Contact

Contact information	**E-mail**
During active study participation: [redacted] Safety Contact	[redacted]

After site closure: Chiesi Safety Contact	[redacted]

The Sponsor or designated CRO will report SAEs events to the regulatory authorities in compliance with the timelines and standards of reporting according to local regulations, as well as to the Investigators and Central IRB/EC, if applicable, by MedWatch/CIOMS form. The Investigator (or Sponsor/CRO where required) must inform the IRB per Sponsor instruction upon receipt of the SUSAR notification. With regard to regulations in force for Pharmacovigilance, the Investigator must fulfil his/her obligation according to the law in force in his/her country.

8.2.3.3 Acute Kidney Injury

Episodes of Acute Kidney Injury (AKI) will be considered and reported as adverse events. AKI will be defined by a 1.5-fold or greater increase in serum creatinine from the previous laboratory value within the priors seven days or an increase in serum creatinine by ≥ 0.3 mg/dl (≥26.5 µmol/l) within 48 hours. Sponsor's Medical Expert will work with the investigator to ensure that such changes in renal function are thoroughly evaluated.

8.2.3.4 Pregnancy

Although pregnancy as such is not considered an AE or SAE, within 24 hours of when s/he is made aware of the pregnancy to the [redacted] Safety Contact using the paper Pregnancy Report Form. The [redacted] Safety Contact will inform Chiesi of the pregnancy within one working day of being notified.

The Pregnancy Report Form will be filled as initial report by the Investigator as soon as s/he becomes aware of the pregnancy, and as a follow-up report any time there is a new information, or at least once at each trimester and at the end of the pregnancy, if applicable.

If the pregnancy meets the criteria for immediate classification of a SAE (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital anomaly, birth defect) the Treating Physician should follow the procedure for reporting SAEs.

Any live birth will have a follow up at year one to record the child's development.

If a male patient's female partner is found to be pregnant while the male patient is on active treatment, the same procedure regarding pregnancy reporting is to be followed and the Pregnancy Report Form should be completed.

9. STUDY PROCEDURES AND FLOW CHART

9.1 Study Flow Chart

Visit Number / Activity	Screening	Visits: 1 to 209	Visits: 3 and 10	Visits: 20, 33, 46 ,60, 72, 86, 99, 112,124, 137, 150, 163, 176, 189, and 202	Visit: 7	Visits: 14, 40, 66, 92,118, 144, 170 and 196	Visit: 20	Visits: 27,79, 131 and 183	Visits: 53, 105, , 157 and 209 or Last Visit
Sign IC	X								
Inclusion/Exclusion Criteria	X								
Physical Examination	X[1]				X[3]	X		X	X
Body Weight	X[1]					X		X	X
Vital Signs	X[1]	X							
Current Medications / Pain Medications / Pre-Medications Use	X[1]	X							
Review of Adverse Events	X[1]	X							
Urine Protein/Creatinine Ratio (UPCR)	X[1]				X[3]	X		X	X
Local Hematology	X[1]				X[3]	X		X	X
Central Biochemistry[4]	X[1]				X[3]	X		X	X
Serum Creatinine and Cystatin C[4]	X[1,7]		X[3]		X[3]	X	X[3]	X	X
Urinalysis – dipstick[4]	X[1]				X[3]	X		X	X
Urine pregnancy test[8]	X[1]				X[3]	X		X	X

Visit Number / Activity	Screening	Visits: 1 to 209	Visits: 3 and 10	Visits: 20, 33, 46 ,60, 72, 86, 99, 112,124, 137, 150, 163, 176, 189, and 202	Visit: 7	Visits: 14, 40, 66, 92,118, 144, 170 and 196	Visit: 20	Visits: 27,79, 131 and 183	Visits: 53, 105, , 157 and 209 or Last Visit
Mainz Severity Score Index (MSSI)	X[1]							X	X
Short Form Brief Pain Inventory (BPI)	X[1]					X		X	X
Anti-Drug Antibodies (IgG)[4]	X[1]		X[3]		X[3]	X	X[3]	X	X
Electrocardiography (ECG)	X[1]				X[3]	X		X	X
Quality of Life (EQ-5D-5L)	X[1,7]					X		X	X
Cardiac MRI or Echocardiogram (for patients that receive a waiver for the Cardiac MRI)	X[1]							X	X
Cardiac function (Stress test)	X[1]							X	X
Brain MRI	X[1]								X
Plasma Lyso-Gb3[4]	X[1]				X[3]	X	X[3]	X	X
Plasma Gb3 concentration[4]					X[3]	X	X[3]	X	X
Follow-up call[5]				X	X[6]				
Study Drug IV Infusion		X[2]							

Note: The flow chart was built to outline the assessments expected per visit and to cover a treatment period potentially up to 98 months (209 visits). The 98 months is estimated to cover commercial availability in all countries participating in the study (per end of study in section 6.1).

[1] Screening visit will happen at the last visit of the previous study; all information will be copied to protocol PB-102-F60.

[2] The first infusions of patients enrolled from PB-102-F20 will be performed at the site according to Appendix 6 to evaluate the tolerability of the infusions and discontinuation of pre-medications if used.

[3] Only applicable for patients who completed PB-102-F20 study.

[4] Will be performed pre-infusion.

[5] A follow-up call should take place with the patients on the Home Care program.

[6] Phone call at Visit 7 applicable only for patients who completed PB-102-F30 and PB-102-F03 studies.

[7] Patients who complete PB-102-F03 need to perform in addition to the procedures from the last visit at the study (Visit 131 or rollover visit) - Sample for serum creatinine and Cystatin C and Quality of life.[8] Females of childbearing potential only

9.2 Study Visits

9.2.1 Screening Visit (Visit 53 of PB-102-F20 Study / Visit 27 of PB-102-F30 Study / Last Visit of PB-102-F03 Study)

- Confirm the patient's eligibility by reviewing the inclusion/exclusion criteria.
- Obtain written informed consent from the patient

Patient ID will be identical to the one used in the previous study.

All information from Visit 53 of PB-102-F20 study, Visit 27 of PB-102-F30 study, or the last Visit of PB-102-F03 will be copied by the system to the Screening visit of CLI-06657AA1-04 (previously PB-102-F60) study.

Patients who completed PB-102-F03 study need to perform at the screening visit the following additional assessments:

- A sample for serum creatinine and Cystatin C.
- Quality of life (Appendix 7. EQ-5D-5L).

9.2.1.1 Visit 53 of PB-102-F20 Study and Missed Assessments Due to COVID-19 Pandemic

Due to the COVID-19 Pandemic local restrictions or patient-related issues, some of the assessments done at Visit 53 of the PB-102-F20 Study could be postponed. In such cases, the assessments will be completed during PB-102-F60 Study. In case the delay in performing the assessments is more than two months into CLI-06657AA1-04 (previously PB-102-F60) Study, the case should be discussed with the Sponsor's Medical Expert to decide if whether or not to complete these assessments at a later time.

9.2.2 Visits 1-209 (Infusions, ± 3 Days)

Pegunigalsidase alfa will be administered by IV infusion every two weeks (±3 days) until commercially available to the patient (approximately 209 infusion visits). Refer to Appendix 1 for the calculation of patient infusion rates.

For patients enrolled from PB-102-F20 study, initial infusions will be managed to evaluate the tolerability and the need for premedication, if used, while in PB-102-F20 study. Therefore, the first infusion of pegunigalsidase alfa will be administered intravenously over 3 hours with 2 hours of post dosing clinical observation. From the second infusion onwards, the infusion will be managed according to Appendix 6, PI evaluation.

With the first infusions, premedication, if used as part of PB-102-F20 infusions, will be continued and then tapered down at the Investigator's discretion pending the patient's tolerability and according to Appendix 6.

The patient will return to the previous treatment set up, either home infusion or predefined infusion center, once the Investigator agrees that it is safe to do so.

Patients who completed PB-102-F30 Study can continue the infusions at the same duration achieved in PB-102-F30 but not less than 60 minutes, with a post-dosing clinical observation time of an additional 60 minutes and the same premedication if used at PB-102-F30.

Patients who completed at least 48 months in PB-102-F03 Study can continue the infusions at the same duration achieved in PB-102-F03 but not less than 60 minutes, with post-dosing clinical observation time of an additional 60 minutes and the same premedication if used at PB-102-F03.

Reduction of the infusion time from 90 to 60 minutes will be conducted in a step-wise manner pending tolerability.

At each visit, all the following activities will be performed:

1. Infusion of the study drug.
2. Collection of new concomitant medications and:
    - Review of ongoing medications.
    - Review of pain medication.
    - Review of premedication.
3. Collection of any information on new and review ongoing AEs.
4. Vital signs will be evaluated before starting the infusion, every 30 minutes during the first hour of infusion, then every 60 minutes until the end of the clinical observation time, and at the end of the observation time.
5. The injection site will be evaluated for any signs of reactivity to the infusion.
6. After reducing the infusion time, patients will be observed clinically for a minimum of one hour post-dosing.

9.2.3 Visits 3 and 10 (±3 d), Applicable for Patients Enrolling from PB-102-F20 Study

- Laboratory tests (pre-infusion):
    - Anti-pegunigalsidase alfa antibodies.
    - Sample for serum creatinine and Cystatin C.
- All activities related to the infusion as described in Section 9.2.2.

9.2.4 Visits: 20, 33, 46, 60, 72, 86, 99, 112,124, 137, 150, 163, 176, 189, and 202 (±6 d)

Follow-up call applicable for patients in the Home Care program.

9.2.5 Visit 7 (Only Applicable for Patients who Completed PB-102-F20 Study) (± 6 Days)

- Physical examination.
- ECG.
- Laboratory tests (pre-infusion):
  - Hematology– local laboratory.
  - Biochemistry- Central laboratory.
  - Urinalysis– dipstick, local laboratory and urine pregnancy test, if female of childbearing potential.
  - Plasma Lyso-Gb3 concentration.
  - Plasma Gb3 concentration.
  - Urine protein/creatinine ratio (UPCR), spot urine sample.
  - Anti-pegunigalsidase alfa antibodies.
  - Sample for serum creatinine and Cystatin C.
- A phone call will be performed at this visit only for patients who completed PB-102-F30 and PB-102-F03 studies.
- All activities related to the infusion as described in Section 9.2.2.

9.2.6 Visits 14, 40, 66, 92,118, 144, 170 and 196 (±3 d)

- Physical examination.
- Bodyweight.
- ECG.
- BPI (Appendix 5. Brief Pain Inventory- BPI (Short Form)).
- Quality of life (Appendix 7. EQ-5D-5L).
- Laboratory tests (pre-infusion):

- Hematology– local laboratory.
- Biochemistry–Central laboratory.
- Urinalysis – dipstick, local laboratory and urine pregnancy test, if female of childbearing potential.
- Plasma Lyso-Gb3 concentration.
- Plasma Gb3 concentration.
- Urine protein/creatinine ratio (UPCR), spot urine sample.
- Anti- pegunigalsidase alfa antibodies.
- Sample for serum creatinine and Cystatin C.

- All activities related to the infusion as described in Section 9.2.2.

9.2.7 Visits 20 (±3 d) Applicable for Patients Enrolling from PB-102-F20

- Laboratory tests (pre-infusion):
  - Anti-pegunigalsidase alfa antibodies.
  - Sample for serum creatinine and Cystatin C.
  - Plasma Lyso-Gb3 concentration.
  - Plasma Gb3 concentration.
- All activities related to the infusion as described in Section 9.2.2.

9.2.8 Visits 27,79, 131 and 183 (± 6 Days)

- Physical examination.
- Bodyweight.
- ECG.
- BPI (Appendix 5. Brief Pain Inventory- BPI (Short Form)).
- MSSI (Appendix 4. The Mainz Severity Score Index (MSSI)).
- Quality of life (Appendix 7. EQ-5D-5L).

- Cardiac MRI or Echocardiogram for patients that have a waiver not to perform an MRI.
- Stress test for cardiac function.
- Laboratory tests (pre-infusion):
  - Hematology– local laboratory.
  - Biochemistry– Central laboratory.
  - Urinalysis – dipstick, local laboratory and urine pregnancy test, if female of childbearing potential.
  - Plasma Lyso-Gb3.
  - Plasma Gb3 concentration.
  - Urine protein/creatinine ratio (UPCR), spot urine sample.
  - Anti- pegunigalsidase alfa antibodies.
  - Sample for serum creatinine and Cystatin C.
- All activities related to the infusion as described in Section 9.2.2.

9.2.9 Visits 53, 105, 157 and 209 (± 6 Days)

- Physical examination.
- Bodyweight.
- ECG.
- BPI (Appendix 5. Brief Pain Inventory- BPI (Short Form)).
- MSSI (Appendix 4. The Mainz Severity Score Index (MSSI)).
- Quality of life (Appendix 7. EQ-5D-5L).
- Cardiac MRI or Echocardiogram for a patient that has a waiver not to perform an MRI.
- Stress test for cardiac function.
- Brain MRI.
- Laboratory tests (pre-infusion):

- Hematology– local laboratory.
- Biochemistry– Central laboratory.
- Urinalysis – dipstick, local laboratory and urine pregnancy test, if female of childbearing potential.
- Plasma Lyso-Gb3 concentration.
- Plasma Gb3 concentration.
- Urine protein/creatinine ratio (UPCR), spot urine sample.
- Anti- pegunigalsidase alfa antibodies.
- Sample for serum creatinine and Cystatin C.

- All activities related to the infusion as described in Section 9.2.2.

9.2.10 Premature Withdrawal Visit

All attempts should be made to perform all the tests for the last visit (Visit 209) for patients terminating the study early. At the withdrawal visit, an infusion will not be performed.

9.2.11 Unscheduled Visit

Unscheduled visit was created for the documentation of assessments or study activities that due to different reasons are not performed in the time window defined by the protocol.

10. STATISTICAL METHODS PLANNED AND SAMPLE SIZE

A detailed statistical analysis plan (SAP) containing the analysis information in detail including the definition of the analysis populations, derivation of variables, convention of analysis scope, and statistical methodology for the analyses of safety and efficacy parameters of pegunigalsidase alfa based on the data collected per the study protocol will be issued prior to the data base lock. In case of disagreement between the SAP and the Clinical Study Protocol, the SAP prevails.

10.1 Determination of Sample Size

This extension study's sample size depends on the number of patients who completed PB-102-F20 study PB-102-F30 study or PB-102-F03 and enrolled into this study; up to 110 male and/or female patients.

10.2 Patient Populations

10.2.1 Intent-to-Treat Population

Intent-to-treat (ITT) population is defined as patients who received any dose (including a partial dose) of study medication.

10.2.2 Per Protocol Population

The Per Protocol (PP) population is defined as patients who complete the study with no major protocol violations.

10.2.3 Safety Population

The safety population is defined as all patients who received at least one dose (partial or complete) of the study medication.

10.3 Analysis

10.3.1 Vital Signs and Physical Examination

For physical examination and vital signs, the frequency count of normal or abnormal by treatment will be tabulated for each body system.

10.3.2 Medications

Summary and/or data listings of the prior, concomitant medication and class of medication will be provided.

10.3.3 Efficacy Variables

For each efficacy variable, the actual and percent change from baseline to each visit the specific variable is measured will be calculated and summarized with descriptive statistics (n, mean and its standard error, median, standard deviation, range, and interquartile range).

eGFR is calculated from the serum creatinine according to the CKD-EPI formula:

$$eGFR\ (ml/min/1.73\ m^2) = 141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018\ [\text{if female}]$$

$$* 1.159\ [\text{if black}]$$

Scr = serum creatinine; $\kappa = 0.7$ for females and 0.9 for males; $\alpha = -0.329$ for females and -0.411 for males, min indicates the minimum of Scr/κ or 1. max indicates the maximum of Scr/κ or 1.

10.4 Safety Analysis

Safety will be assessed by evaluating AEs, injection site reactions, ECG, clinical laboratory results, brain MRI, anti-pegunigalsidase alfa antibodies ability to taper off infusion pre-medication, and requirement for using premedication to manage infusions reactions.

10.4.1 Adverse Events

AEs will be coded to system organ class and preferred term using a medical dictionary for regulatory activities (MedDRA) version 19.0. All AEs occurring after the study treatment (TEAEs) will be reported, including events present at baseline that worsened during the study.

AEs will be summarized with respect to the incidence of AEs (the number of patients reporting at least one episode of a specific AE), the incidence of AEs by severity within the body system, the incidence of AEs by attribution within the body system, and incidence of AEs causing withdrawal and incidence of SAEs. Regarding severity and attribution summaries, the most extreme outcome (highest severity and closest to study drug-related) will be used for those patients who experience the same AE on more than one occasion.

Hypersensitivity reactions will be analyzed as AEs of particular interest using descriptive statistics.

Written narratives will be provided for all serious, unexpected, or other significant AEs judged to be of particular interest because of their clinical importance.

10.4.2 Clinical Laboratory

Clinical Laboratory examination will be performed prior to treatment and during treatment.

Descriptive statistics will be presented for the value and abnormality of each of the clinical laboratory results by visit. Shift tables describing abnormality shifts from baseline to after treatment and follow-up will be created.

10.5 Interim Analysis

Interim analyses may be conducted for administrative purposes.

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Source Data and Records

Source data are all the information in original records and certified copies of original records of clinical findings, observations, laboratory reports, data sheets provided by the Sponsor, or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IRB review(s), and regulatory inspection(s), with direct access to all the required source records.

All study records will be retained for a period of time as defined by the regulatory authority for the country in which the investigation is conducted. Generally, this means at least two years following the date on which the drug is approved by the regulatory authority for marketing for the purposes that were the patient of the investigation. In other situations (e.g., where the investigation is not in support of or as part of an application for a research or marketing permit), a period of 2 years following the date on which the entire clinical program is completed, terminated or discontinued, or the investigational application under which the investigation is being conducted is terminated or withdrawn by the regulatory authorities.

In case the Investigator retires, relocates, or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records may be transferred to any other person who will accept responsibility for the records. Notice of such a transfer must be given in writing to the Sponsor. The Investigator must contact the Sponsor prior to disposal of any records related to this study.

11.2 Reporting of Results

The CRF is an integral part of the study and subsequent reports. Therefore, the CRF must be used to capture all study data recorded in the patient's medical record. In addition, the CRF must be kept current to reflect patient status during the study. Only a patient screening and treatment number will be used to identify the patient

The monitor is responsible for performing on-site monitoring at regular intervals throughout the study to verify adherence to the protocol; verify adherence to local regulations on the conduct of clinical research; and ensure completeness, accuracy, and consistency of the data entered in the CRF.

Chiesi or their designated CRO will monitor completed CRFs. A case report form will be provided for each screened patient.

All protocol-required information collected during the study must be entered by the Investigator, or designated representative, in the [redacted] e*CRF™, an Internet-based electronic data collection system. All details of the CRF completion and correction will be explained to the investigator. The management module of [redacted] e*CRF™ includes edit check and query systems that seamlessly integrate with the data entry system. All modifications to the data in the eCRF are tracked by an electronic audit trail (the date and identity of the person making the change are instantaneously recorded). [redacted] is 21CFR Part 11 compliant.

If the Investigator authorizes other persons to make entries in the eCRF, these persons' names, positions, and signatures must be supplied to the Sponsor.

The Investigator, or designated representative, should complete the eCRF as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or other study procedure. Any outstanding entries must be completed immediately after the final examination. In addition, an explanation must be provided for all missing data, altered data, and/or out-of-range data by design.

The completed case report form must be reviewed and signed by the Investigator named in the study protocol or by a designated sub-investigator.

Final monitored and audited eCRFs will be provided by the Sponsor to the sites at the end of the study in a PDF file format.

11.3 Confidentiality of Patient Data

The investigator will ensure that the confidentiality of the patients' data will be preserved. In the CRF or any other documents submitted to the Sponsor, the patients will not be identified by their names but by an identification system consisting of a number in the study. In addition, the investigator will maintain documents not meant for submission to the Sponsor, e.g., the confidential patient identification code and the signed informed consent forms, in strict confidence.

12. REPORTING AND PUBLICATION

12.1 Confidentiality of Study Data

Any information relating to the study product or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The investigator and any other persons involved in the study will protect the confidentiality of this proprietary information belonging to Chiesi Farmaceutici S.p.A.

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14. APPENDICES

14.1 Appendix 1. Tolerability and Infusion Rate Algorithm

The infusion rate (IR) may be adjusted according to individual patient symptoms and signs. The assumptions with respect to adverse experiences to the infusion are:

1. Most of the patients will tolerate the infusion without any unusual symptoms or events.

2. Patients presenting symptoms and signs of **severe** hypersensitivity will be evaluated according to the CTCAE Drug Toxicity criteria, and there may be discontinuation of treatment according to the protocol.

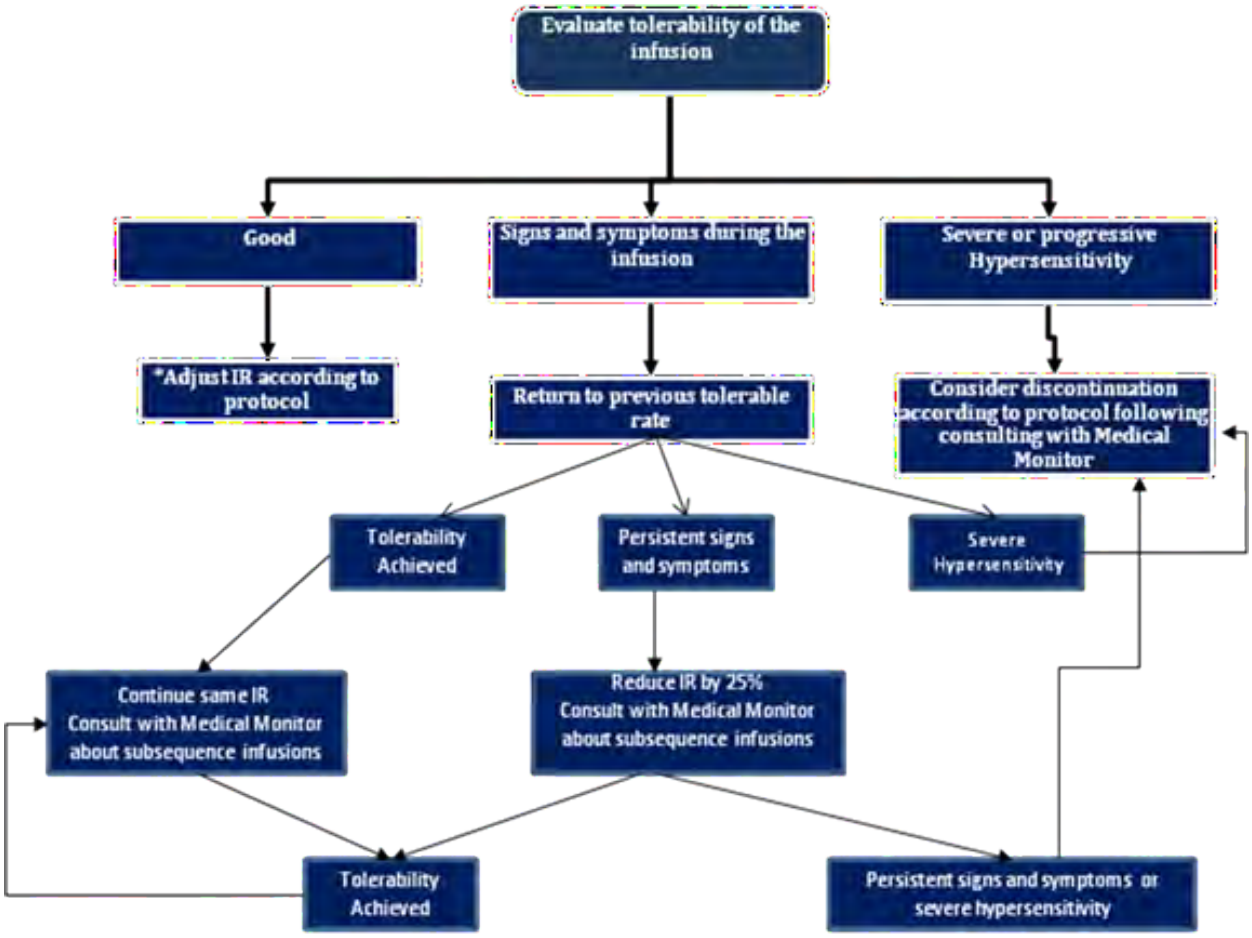
3. Patients may present signs and symptoms that will respond to reducing the infusion rate and may not appear at the next infusion.

4. Tolerability and the patient-specific infusion rate will be assessed and decided by the Investigator according to the patient's vital signs and clinical status.

Definitions to be applied regarding the tolerability of infusions are as follows:

Good Tolerability	Partial Tolerability	Poor Tolerability
The infusion was performed without signs and symptoms (such as burning, pruritus, flushing, discomfort, or change in vital signs).	Signs and symptoms appeared during the infusion **and resolved after slowing infusion rate** or at the end of the infusion.	Signs and symptoms meeting the definitions of CTCAE Grade 1 or 2 toxicity responding to reduction of infusion rate or responding to treatment (example, an antihistamine for urticaria).

The specific algorithm for infusion rates to be followed:



Changes in Infusion Rates According to the Protocol:

Signs and symptoms will determine the tolerability of the infusions in these patients during the infusion and observation time.

The infusion rate should be adjusted according to patient tolerability as described above; continuation of the infusion rate should be agreed upon between the PI and the Sponsor's Medical Expert as needed.

14.2 Appendix 2. Pegunigalsidase alfa Hypersensitivity Evaluation and Treatment Algorithm

During and after infusion of pegunigalsidase alfa, the following algorithm will be followed to monitor and manage the occurrence of hypersensitivity, anaphylaxis, or anaphylactoid reactions. The Investigator can follow an alternative treatment algorithm when used by the local institution. Laboratory assessment, described in this appendix, should be collected in any case. Sponsor's Medical Expert will be notified and available for consultation if needed.

14.2.1 Clinical Signs

Early:

- A sensation of warmth and itching
- Feelings of anxiety Moderate
- Pruritus
- Flushing
- Urticaria
- Chest discomfort
- Mild Hypotension Progressive
- Erythematous or massive urticarial rash
- Edema of face, neck, soft tissues Severe
- Hypotension
- Bronchospasm (wheezing)
- Laryngeal edema (dyspnea, stridor, aphonia, drooling)
- Arrhythmias

14.2.2 Treatment Algorithm

With the onset of any of the above clinical signs, immediately discontinue study drug administration and initiate the following monitoring.

1. Continuous electrocardiographic monitoring.
2. Continuous pulse oximetry.
3. Measure blood pressure every 5 minutes
4. Perform chest auscultation every 5 minutes
5. Collect blood samples for Tryptase (29-33), antibodies, and complement C3, C4. Tryptase samples need to be withdrawn at:

- 1st sample to be taken 0.25-3 hours after onset of symptoms.
- 2nd sample to be taken between 3-6 hours.

- 3rd sample to be taken 24-48 hours to verify the return to baseline.

Suspected Hypersensitivity Reactions at Home Care:

In the case that a reaction, suspected to be a result of hypersensitivity, occurs during a home care visit, the following steps need to be performed:

- The study nurse should inform the site and treat the patient according to Investigator's Emergency Treatment Plan.
- The next infusion needs to be scheduled and performed at the site.

14.2.3 Treat as Follows

Urticaria or Edema of the Face, Neck, or Soft Tissues:

- Epinephrine 1:1000 solution, 0.5 mL subcutaneously, repeat as needed every 5-10 minutes.
- Antihistamines.
- Corticosteroids.

Hypotension (Systolic Blood Pressure (SBP) ≤ 90 mmHg):

- Isotonic sodium chloride solution, 1 L every 30 minutes as needed to maintain SBP > 90 mmHg.
- Epinephrine 1:10,000 solution given IV at 1 µg/minute initially, then 2-10 µg/minute to maintain SBP > 90 mmHg.
- Norepinephrine 4 mg in 1 L 5% dextrose in water given IV at 2-12 µg/min to maintain SBP> 90 mmHg.
- Glucagon 1 mg in 1 L 5% dextrose in water give IV at 5-15 µg/minute for refractory hypotension.

Bronchospasm:

- Oxygen by face mask at 6-8 L/minute to maintain oxygen saturation at > 90%.
- Epinephrine 1:1000 solution, 0.5 mL subcutaneously.
- Albuterol 0.5 mL of 0.5% solution, in 2.5 mL of sterile saline, every 15 minutes, up to three doses.
- Inhaled beta-agonists.
- Corticosteroids Laryngeal edema.
- Epinephrine 1:1000 solution, 0.5 mL subcutaneously, repeat as needed every 5 to 10 minutes.

- Corticosteroids.

If symptoms resolve within a single study visit and the investigator determines the symptoms were not an occurrence of progressive or severe hypersensitivity, anaphylaxis, or anaphylactoid reactions, then administration of the drug may continue according to the algorithm provided above, and at the discretion of the Investigator.

14.2.4 Premedication

Premedication for subsequent PRX-102 infusions may be considered at the investigator's discretion for patients experiencing early clinical signs of hypersensitivity or rash/urticaria that respond promptly to oral antihistamine administration (see for adjustment of infusion rate). The premedication will be administered according to the following steps as needed to prevent progressive hypersensitivity:

1. Antihistamine (H1 blocker: diphenhydramine, hydroxyzine, cetirizine, loratadine, desloratadine) at a standard dose, 12 hours and 2 hours before starting of the infusion.
2. H1 blocker plus H2 blocker (ranitidine, cimetidine, famotidine) at standard doses 12 hours and 2 hours before the start of the infusion.

H1 blocker plus H2 blocker plus prednisone up to 50 mg administered 12 hours and 2 hours before the start of the infusion.

14.3 Appendix 3. Cardiac MRI

14.3.1 Patients and Sites

For each cardiac MRI to be conducted throughout the study, the test will be performed after the Investigator reviews the patient's kidney function.

For patients who may present one of the following conditions, the performance of the Cardiac MRI may be discussed with the Sponsor's Medical Expert as needed:

- GFR < 30 mL/min/1.73m2 based on the last serum creatinine value, or
- Suspected AKI

Gadolinium Warnings from USPI

WARNING: NEPHROGENIC SYSTEMIC FIBROSIS (NSF)
See full prescribing information for complete boxed warning

Gadolinium-based contrast agents (GBCAs) increase the risk for NSF among patients with impaired elimination of the drugs. Avoid use of GBCAs in these patients unless the diagnostic information is essential and not available with non-contrasted MRI or other modalities.

- **The risk for NSF appears highest among patients with:**
  - **Chronic, severe kidney disease (GFR < 30 mL/min/1.73m^2), or**
  - **Acute kidney injury.**
- **Screen patients for acute kidney injury and other conditions that may reduce renal function. For patients at risk for chronically reduced renal function (for example, age >60 years, hypertension or diabetes), estimate the glomerular filtration rate (GFR) through laboratory testing (5.1).**

14.3.2 Magnetic Resonance Imaging Data

Each patient enrolled in this trial will have a cardiac MRI at the Screening Visit (Visit 53 in PB-102- F20, Visit 27 in PB-102-F30, or at the last visit of PB-102-F03 study) in order to reference further MRI evaluations in the study. A set of ECG gated cine and delayed contrast-enhanced MRI sequences (SSFP resp. Inversion recovery Gradient echo) will be acquired. A gadolinium-based contrast agent will be used during image acquisition of the delayed contrast-enhanced scan.

The sequences will be defined based on the equipment and ability to provide sufficient image quality and contrast for myocardium and fibrosis detection and quantification in Fabry's disease patients.

14.3.3 MRI Evaluation Parameters

The following MRI parameters will be evaluated during this trial:

- Number and location of left ventricular segments with fibrosis.
- Percentage and mass of cardiac fibrosis (in grams).
- Left ventricular myocardial mass (in grams).
- Left ventricular myocardial mass index.
- Left ventricular ejection fraction.

14.3.4 Sites and Image Data Management

All image management activities will be centralized and conducted by an independent imaging Contract Research Organization (CRO) with operational capabilities in Europe and the United States in compliance with all regulatory requirements. An overview of the main activities performed by the imaging CRO is provided in the following sections.

14.3.4.1 Standardization of Image Acquisition, Initial Site Qualification

This is an extension study which includes patient from PB-102-F20, PB-102-F30 and PB-102-F03 studies. The imaging during PB-102-F60 study will be performed in the same facilities approved for the previous studies.

14.3.4.2 Subject Sedation

Subjects may require sedation in order to obtain the high-quality images required. Sites may use standard sedation protocols approved by the institution.

14.3.4.3 Quality Control of Image Data and Site Quality Assurance

The image data will be collected, and quality controlled by the imaging CRO for checking the technical adequacy, the compliance of data acquisition with the study imaging protocol, the anonymization of the images, and the diagnostic quality of the images (their appropriateness for centralized evaluations). If the imaging CRO detects any quality-related issue, specific queries will be sent to the sites to implement appropriate corrective (including potential repeat scans if needed) and preventive actions.

14.3.5 Image Processing and Centralized Analysis

14.3.5.1 Cardiac MRI Assessment

Analysis of the cine short-axis and delayed contrast-enhanced images of the left ventricle will be performed with dedicated MRI quantification software.

Myocardial contours will be detected semi-automatically and manually edited and quality controlled by an expert technician at the imaging CRO.

The left ventricular contours will be submitted for final approval to an independent and blinded reader.

Based on approved contours, the left ventricular mass and % and mass of the fibrotic area are calculated automatically by the software algorithm.

14.3.5.2 Centralized and Blinded Image Review by Independent Readers

The MRI data will be centrally evaluated, in a fully blinded manner, by an independent reader. The reading sessions will be organized at the imaging CRO site. The same image evaluation procedure will be used for all patients' MRI scans in this trial.

14.3.5.3 The Expertise of Independent Readers, Training Sessions

The reader will be a Cardiologist with a significant experience in cardiac MRI. The reader will be trained prior to the start of centralized image review sessions.

He/she will be provided with a Read Rules document and will be given training on using the software. Test cases representing non-study Fabry patients will be used for the training. Main consensus issues (contour detection in apical and basal LV slices, trabeculae and papillary muscles, the threshold for delayed enhanced areas, etc.) will be discussed with the reader and documented.

14.3.5.4 Conduct of the Centralized Image Review Sessions

The reader will be fully blinded to, patient ID, and site number. The images will have been pre-analyzed by experienced image analysis technologists from the imaging CRO.

The image review sessions by the cardiologist will include:

14.3.5.5 Efficacy Image Review

MRI analysis results at baseline will act as a reference for further MRI evaluations done in this study.

14.3.6 Data and Report Transfers to Sponsor

- Efficacy image Review sessions will be exported to the Sponsor using a predefined, standardized, and secure data transfer procedure.
- The final Study database will be submitted to the Sponsor in digital format.

14.3.7 Direct Access to Study Data

Direct access to study data will be made possible by the imaging CRO for audit purposes. Such study data include:

- Information related to interactions between the imaging CRO and the sites (Queries, Data Clarification Forms, test data submitted by the sites, etc.)

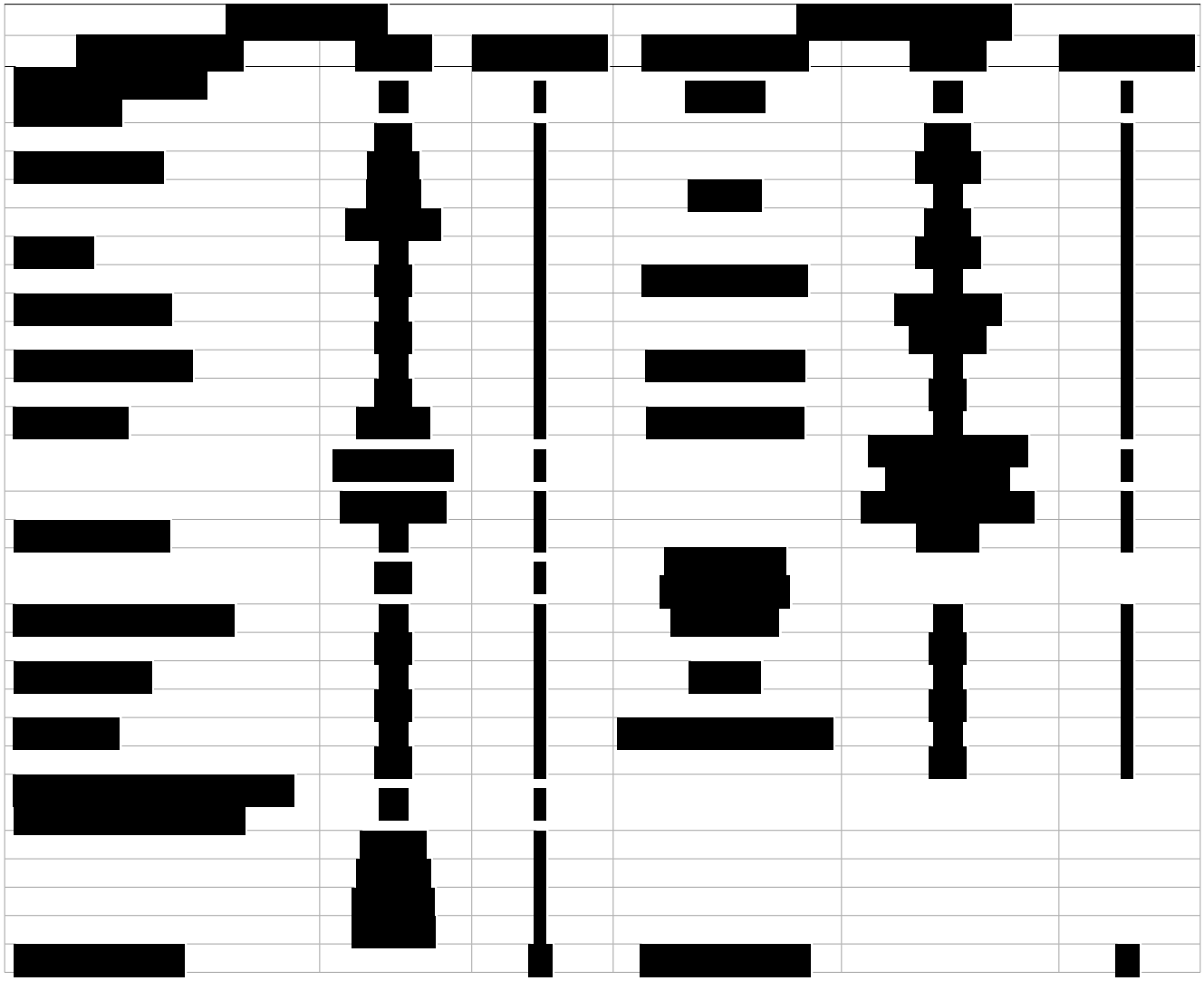
- Native MRI data
- Data processed and generated by the imaging CRO
- Data generated by the blinded reader
- Audit trails

14.3.8 Unevaluable MRI

Unevaluable MRI data can result from a poor-quality image due to patient motion, improper left ventricular coverage, technical problems with the image transmission to the imaging CRO, etc. The imaging CRO procedures for ensuring quality images are meant to reduce or eliminate such poor-quality images (Section 14.4.4.3 above).

If an adequate patient image cannot be obtained for a given time point in the study, the problem with the image will be documented at the imaging CRO. In addition, the imaging CRO will document all attempted corrective actions with the investigative site imaging center.

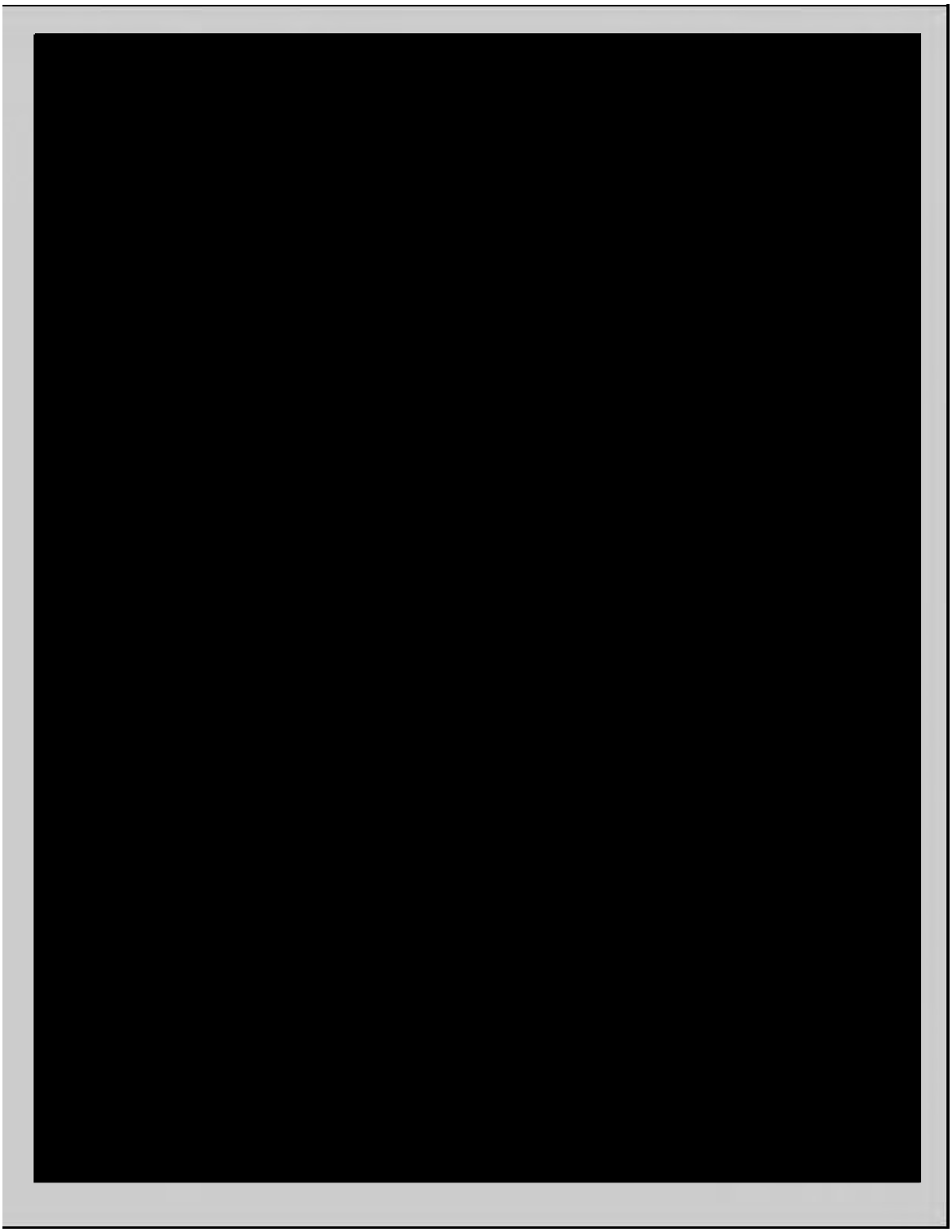
[redacted]

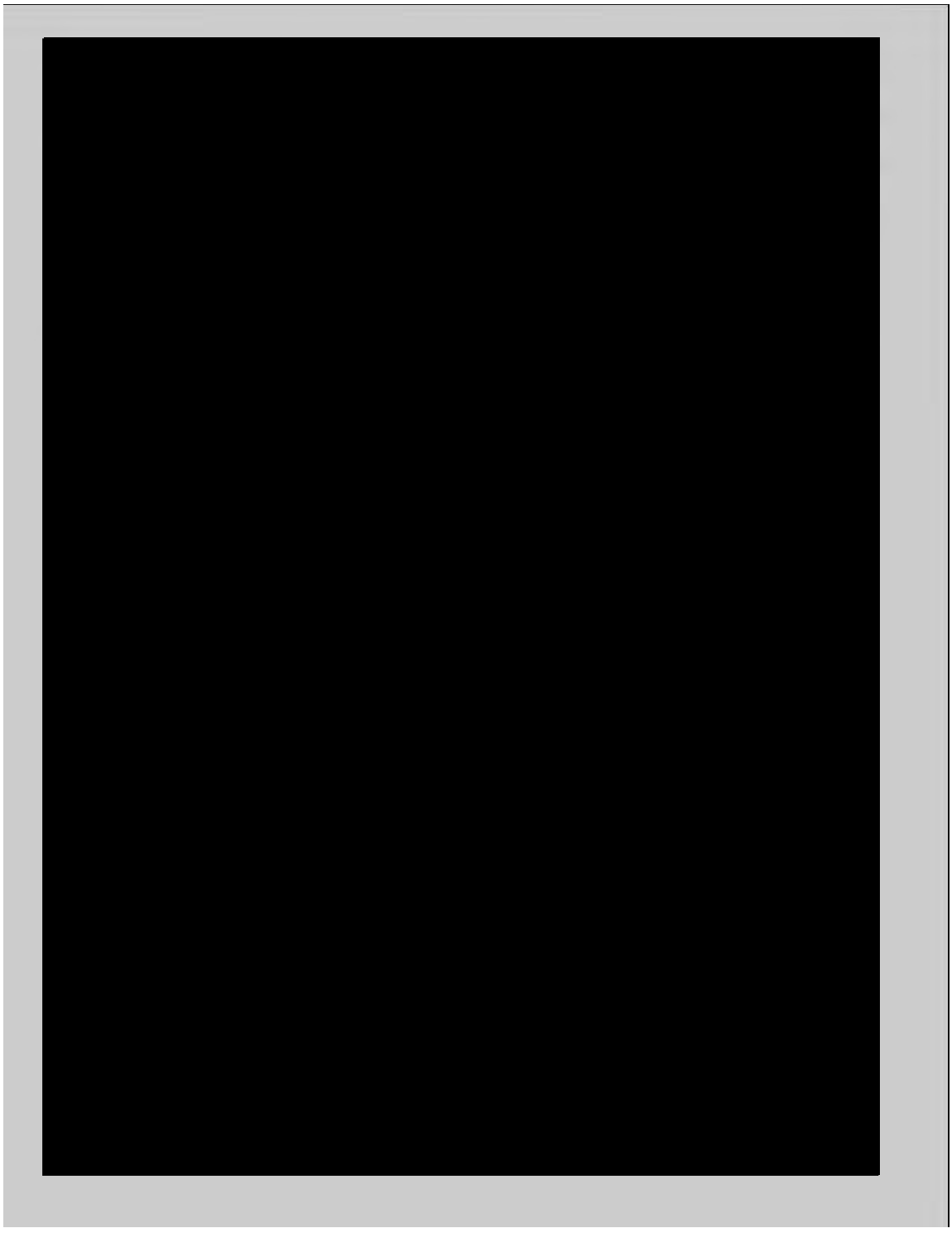


hypertrophy; MRI, magnetic resonance imaging; TIA, transient ischemic attack.

14.5 Appendix 5. Brief Pain Inventory- BPI (Short Form)

(www.mdanderson.org/education-and-research/symptom-assessment-tools/BPI_User_Guide.pdf)
The short form of the BPI will be performed every six months in this study.





14.6 Appendix 6. Guide for the Transition of Infusion Time

Guide for the transition of infusion time and premedication for patients who completed the PB-102-F20 study and enrolled in the PB-102-F60 study.

14.6.1 Introduction

PB-102-F20 is a double-blind study in which patients are treated with pegunigalsidase alfa or agalsidase beta (Fabrazyme). The following procedures described in this appendix were designed to keep the blindness of PB-102-F20 study, which is ongoing while recruiting patients to PB-102-F60 study.

The patients completing PB-102-F20 study can be divided into four different groups based on the infusion procedures:

A. Patients whose infusions duration is 1.5 hours and are not treated with premedications.
B. Patients whose infusions duration is 1.5 hours and are treated with premedications.
C. Patients whose infusions duration is > 1.5 hours and are not treated with premedications.
D. Patients whose infusions duration is > 1.5 hours and are treated with premedications.

First infusions should be implemented according to the following guidelines. Then changes in infusion rate need to be implemented according to the patient tolerability.

A. Patients whose infusions duration is 1.5 hours and are not treated with premedications:

1. The first infusion will be administered over 3 hours.
2. If the tolerability is acceptable to the investigator, the second infusion length could be reduced to 1.5 hours.
3. If the tolerability is uncertain, the reduction of the infusion time will be made stepwise pending tolerability. The time of infusion can be decreased by 30 minutes every third infusion, up to a minimum infusion time of 1.5 hours or best tolerable infusion time achieved under the agreement of the Investigator.
4. After achieving an infusion length of 1.5 hours, the infusion length can be further decreased to 1 hour (60 minutes) pending patient tolerability.

B. Patients whose infusions duration is 1.5 hours and are treated with premedications:

1. The first infusion will be administered over 3 hours.
2. After the first infusion, an attempt to reduce premedications will be made stepwise at the infusion rate of 3 hours.
3. If the tolerability is acceptable to the investigator, the next step is to reduce the infusion

length to 1.5 hours in the following infusion.

4. If the tolerability is uncertain, the infusion time reduction will be made step-wise pending tolerability. The infusion time can be decreased by 30 minutes every third infusion up to a minimum infusion time of 1.5 hours or best tolerable infusion time achieved under the agreement of the Investigator.

5. After achieving an infusion length of 1.5 hours, the infusion length can be decreased to 1 hour (60 minutes) pending patient tolerability.

C. Patients whose infusions duration is > 1.5 hours and <u>are not</u> treated with premedications:

1. The first infusion will be administered over 3 hours.

2. If the tolerability is acceptable, infusion # 2 will be administered at the same rate as the last visit of PB-102-F20:

   a. If infusion #2 tolerability is acceptable, infusion #3 can be administered over 1.5 hours.

   b. If infusion #2 tolerability is uncertain, the infusion time reduction will be step-wise pending the patient's tolerability.

The infusion time can be decreased by 30 minutes every third infusion, up to a minimum infusion time of 1.5 hours or best tolerable infusion time achieved and under the agreement of the Investigator.

3. After achieving an infusion length of 1.5 hours, the infusion length can be further decreased to one hour (60 minutes) pending the patient's tolerability.

4. If the patient were infused in PB-102-F20 over 3 hours or more, the patient's infusion management would be discussed with the Investigator and Sponsor's Medical Expert if needed.

D. Patients whose infusions duration is > 1.5 hours and are treated with Premedication:

1. The first infusion will be administered over 3 hours.

2. After the first infusion, reducing the premedication will be made stepwise and pending the patient's tolerability over 3 hours.

3. After the attempt to reduce premedication, if the tolerability is acceptable, the next infusion will be administered for the same rate given at the last PB-102-F20's visit:

   a. If the tolerability is acceptable, the following infusions can be administered over 1.5 hours.

b. If the tolerability is uncertain, the infusion time reduction will be made stepwise, pending the patient's tolerability. The infusion time can be decreased by 30 minutes every third infusion, up to a minimum infusion time of 1.5 hours or optimal tolerated infusion time achieved, under the agreement of the Investigator.

4. After achieving an infusion length of 1.5 hours, the infusion length can be further decreased to one hour (60 minutes) pending the patient's tolerability.

5. If the patient were infused at PB-102-F20 over 3 hours or more, the patient's infusion management can be discussed with the Sponsor's Medical Expert.

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