Statistical Analysis Plan for Final Analysis
Ver 2.0

Protocol PB-102-F20
Version 5, July 14, 2017

A Randomized, Double Blind, Active Control Study of the Safety and Efficacy of Pegunigalsidase Alfa Compared to Agalsidase Beta on Renal Function in Patients with Fabry Disease Previously Treated with Agalsidase Beta

Sponsor: Protalix Ltd.

Prepared by: Anat Sakov, PhD., DataSights Ltd.

Approval

Name	Signature	Date
Sponsor: Raul Chertkoff, M.D., E.M.B.A. Vice President Medical Affairs	DocuSigned by: Jan-30-2022 | 14:51 ISST Signer Name: Raul Chertkoff Signing Reason: I approve this document Signing Time: Jan-30-2022 | 14:51 ISST 0E9E9C392F27436BA1BBBF357A67B1FC	
Statistician: Anat Sakov, PhD. DataSights Ltd.	A. Sakov	Jan 27, 22

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abbreviations

ACE	Angiotensin converting enzyme
ADA	Anti-drug antibody
AE	Adverse event
AKI	Acute kidney injury
ANCOVA	Analysis of covariance
ARB	Angiotensin receptor blocker
AUC_{0-t}	Area under the concentration-time curve from Baseline to a specified time (t)
$AUC_{0-\infty}$	Area under the concentration-time curve from Baseline to infinity
BDR	Blinded data review
BLA	Biologics license application
BPI	Brief pain inventory
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	Maximum concentration observed
CRO	Clinical Research Organization
CSR	Clinical study report
DBL	Database lock
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EMA	European medicinal agency
EQ-5D-5L	5-level EuroQol 5-Dimension Questionnaire
FCE	Fabry clinical event
FD	Fabry Disease
FDA	United States Food and Drug Administration
Gb3	Globotriaosylceramide
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCG	Human chorionic gonadotropin
HIV	H immunodeficiency virus
IC	Informed consent
IgG	Immunoglobulin G
IRR	Infusion related reaction

ITT	Intention to treat
IV	Intravenous
KD	Kidney disease
KM	Kaplan-Meier
LPLV	Last patient last visit
LVM	Left ventricular mass
LVMI	Left ventricular mass index
Lyso-Gb3	Globotriaosylsphingosine
MAA	Marketing authorization application
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed model repeated measures
MNAR	Missing not at random
MRI	Magnetic resonance imaging
MSSI	Mainz Severity Score Index
PK	Pharmacokinetics
PP	Per protocol
PrT	prothrombin time
PT	Preferred term
PTT	Partial thromboplastin time
SAP	Statistical analysis plan
SD	Standard deviation
SE	Standard error
SOC	System organ class
SOP	Standard operating procedure
$t_{1/2}$	Half-life
TEAE	Treatment-emergent adverse event
TLF	Tables, listings, and figures
T_{max}	Time at which the concentration is the maximum value (C_{max})
UPCR	Urinary protein to creatinine ratio
US	United States

1. INTRODUCTION

This statistical analysis plan (SAP) contains detailed information on the definition of the analysis populations, derivation of variables, conventions of analysis scope, and statistical methodology for the analyses of efficacy and safety of pegunigalsidase alfa (also known as PRX-102) and agalsidase beta administered by intravenous (IV) infusion based on data collected per protocol PB-102-F20, a Phase 3 study sponsored by Protalix Ltd. Should the SAP and the protocol be inconsistent with respect to the planned analyses, the language of the SAP is governing.

The pharmacokinetic (PK) analysis is not within the scope of this SAP and is detailed in a standalone PK analysis plan.

A pre-planned interim analysis occurred when the last patient completed the 12-month visit to support a Marketing Authorization Application (MAA) submission to the European Medicines Agency (EMA). The final analysis will occur when the last patient completes the 24-month visit and will support a biologics license application (BLA) re-submission to the FDA.

The SAP that was signed on April 15th, 2021 prior to Data Base Lock (DBL) and unblinding for the interim analysis, included non-inferiority of PRX-102 compared to agalsidase beta for the interim analysis and superiority of PRX-102 compared to agalsidase beta for the final analysis. However, following the conversion of agalsidase beta to a full approval in March 2021, it was agreed with the FDA (End-of-Review Meeting, Sep 9th, 2021) that it is not necessary anymore to demonstrate superiority over agalsidase beta. A Type C meeting to reach agreement with the FDA on the proposed primary model to assess non-inferiority took place in January 21, 2022 and the SAP reflects this discussion.

In addition, analysis that were conducted as post-hoc after the interim analysis are added to the SAP, as well as some other minor changes (all are documented). None of the analyses performed at the interim analysis will be removed but following a comment by FDA, covariates were changed.

As this SAP is now only for the final analysis, sections/paragraphs relating to the interim analysis were deleted and/or updated to include only the final analysis.

In light of the unplanned conversion of agalsidase beta to a full approval and the change in regulatory requirements following that, some changes had to be made also to the unblinding plan.

Section 13 describes the changes from the protocol to the interim analysis SAP and from the interim analysis SAP to the final analysis SAP.

Any deviations from this SAP during the actual data analysis will be documented properly in the final Clinical Study Report (CSR). The SAP will be finalized before the database lock (DBL) for the final analysis.

2. OBJECTIVES AND ENDPOINTS

2.1 Objectives

The objective of this study is to evaluate the safety and efficacy of PRX-102 compared to agalsidase beta in Fabry disease patients with impaired renal function.

2.2 Endpoints

2.2.1 Primary Efficacy Endpoint –Interim and Final Analyses

The primary efficacy endpoint is the mean annualized change (slope) in the estimated glomerular filtration rate ($eGFR_{CKD-EPI}$). For simplicity, for the remainder of this document the subscript of CKD-EPI will be omitted.

2.2.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

- Change from baseline to all time points in the following measures:
  - Plasma globotriaosylsphingosine (Lyso-Gb3)
  - Left Ventricular Mass Index (LVMI) (g/m^2) by Magnetic Resonance Imaging (MRI)
  - Plasma globotriaosylceramide (Gb3)
  - Urine Lyso-Gb3
  - Protein/Creatinine ratio spot urine test
  - 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 Clinical Events (FCE)
- Achieving Fabry Kidney Disease therapeutic goals

2.2.3 Pharmacokinetic Endpoints

Blood samples for PRX-102 PK analysis will be taken for up to 30 patients entering the study, of which PK analysis will be evaluated only for patients receiving PRX-102. The following parameters (but not restricted to) will be calculated for them:

- C_{max}
- T_{max}
- AUC0-t
- t1/2
- AUC0-∞

2.2.4 Safety Endpoints

The following safety measures will be evaluated throughout the study:

- Clinical laboratory tests
- Physical examination
- Injection site reactions
- Electrocardiogram (ECG)
- Treatment-emergent adverse events (TEAEs)
- Infusion Related Reactions (IRRs)
- Infusion pre-medication
- Treatment-emergent Anti-Drug Antibodies (ADA)
- Vital signs

3. STUDY DESIGN

The study is a randomized, double blind, active-control study of the efficacy and safety of PRX-102 compared to agalsidase beta in adult Fabry disease patients with impaired renal function under treatment with agalsidase beta for at least one year and on a stable dose for at least 6 months. Patient age will be 18 to 60 years. The estimated glomerular filtration rate (eGFR) at screening using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation will be between 40 to 120 mL/min/1.73 m^2 and evidence of renal disease progression of at least -2 mL/min/1.73 m^2/year will be present. No more than 50% of the patients enrolled will be female.

Patients will be randomized to receive intravenous infusions of PRX-102 1 mg/kg or agalsidase beta 1 mg/kg every two weeks at a 2:1 ratio. Randomization will be stratified by urine protein to creatinine ratio (UPCR) of <1 or ≥ 1 gr/gr by spot urine sample. Infusion duration will initially be 3 hours but may be decreased gradually after establishment of tolerability by agreement of the investigator and Protalix Medical Director.

The primary efficacy endpoint is the mean annualized change (slope) of eGFR over the course of the study as estimated from a longitudinal mixed model. The comparison of the slopes between the two arms will be evaluated after the last patient complete at least 12 months of treatment (interim analysis) and when the last patient complete 24 months of treatment (final analysis). The objective of the interim analysis is to demonstrate non-inferiority with a margin of -3.0 mL/min/1.73 m^2/year and will be used to support the submission of a MAA to the EMA. The objective of the final analysis with the data from 24 months of treatment is to demonstrate superiority and will be part of the confirmatory trial supporting the BLA conversion from Accelerated Approval to traditional approval.

At the end of the study all patients may be offered the opportunity to continue to an open-label extension study (PB-102-F60) with PRX-102 1 mg/kg administered every 2 weeks.

3.1 Sample Size and Statistical Power Consideration

The section below is based on the plan at the time of the protocol and does not reflect the change in the planned analysis for the final analysis. The number of patients randomized was 78, as planned.

This orphan indication allows for a small number of patients, given the rarity of the disease and the difficulties in allocating patients for clinical trials. Two tests are planned to be performed as part of the primary efficacy analysis for this protocol. The first test, to test the non-inferiority hypothesis, will be performed using data collected after the last patient have at least 12 months of treatment (interim analysis). The second test, to test the superiority hypothesis, will occur with the data from 24 months of treatment (final analysis). We now describe the statistical power/sample size considerations for these two tests.

Sample Size and Power for the Non-Inferiority Analysis (Interim Analysis)

With a total of approximately 66 patients in 2:1 randomization ratio there is at least 90% power to demonstrate the non-inferiority of PRX-102 vs. agalsidase beta in terms of mean annualized change (slope) in eGFR. The power is computed assuming one-sided two-sample t-test with a one-sided alpha level of 0.025 and the non-inferiority margin of -3.0 mL/min/1.73m^2/year. The true difference in slopes is assumed to be 1.1 mL/min/1.73 m^2/year in favor of PRX-102 and the standard deviation of the slopes being 1.5 mL/min/1.73 m^2/year in each group.

Sample Size and Power for the Superiority Analysis (Final Analysis)

With a total of approximately 66 patients in 2:1 randomization ratio there is approximately 80% power to detect a difference of 1.1 mL/min/1.73 m^2/year between the mean annualized change (slope) in the two arms. The power is computed assuming two-sample t-test at two-sided alpha level of 0.05 and the standard deviation of the slopes being 1.5 mL/min/1.73 m^2/year in each group.

For this analysis the null hypothesis is that the difference in mean annualized change in eGFR between the treatment groups is 0 versus an alternative hypothesis that it is not 0.

Based on previous research, the mean annualized change (slope) in eGFR in patients treated with agalsidase beta is -3.0 mL/min/1.73 m^2/year. A 1.1 reduction in the rate of decline in renal function (improvement) is anticipated to be equal to a mean annualized change (slope) in eGFR with PRX-102 of -1.9 mL/min/1.73 m^2/year representing an approximately 30% improvement, which would be considered a clinically relevant improvement.

To allow for a drop-out rate as large as 15%, approximately 78 patients will be randomized. Approximately 52 patients will be randomized to PRX-102 and approximately 26 patients will be randomized to agalsidase beta to have approximately 44 and 22 completers in the PRX-102 and agalsidase beta arms, respectively.

These power calculations are based on two-sample t-test and were performed using PASS-13 Tests for Two Means procedure (Hintze, 2014).

3.2 Randomization

Patients will be randomized in 2:1 ratio to PRX-102 and agalsidase beta. Randomization will be stratified by urinary protein to creatinine ratio (UPCR) of < or ≥ 1 gr/gr (1 mg/mg or 1000 mg/g) by spot urine sample.

3.3 Study Flow Chart

Table 1: Study Assessments

Activity Visit Number	S[4]	1	2 through 52	All odd numbered visits	2, 15, 28 and 2 weeks after Visit 53	4	2, 3, 5, 9, 11	7, 20	14	27	33, 47	40	53
Sign IC	x												
Assign screening number	x												
Inclusion/exclusion criteria	x	x											
Demographics	x												
Medical & Specific FD history	x												
Physical examination	x	x						x	x	x	x	x	x
Body weight	x	x						x	x	x	x	x	x
Body height	x												
Vital signs	x	x	x										
Current medications Pain medications Pre-medication Use	x	x	x										
Alpha-galactosidase activity in plasma	x												
Alpha-galactosidase activity in leucocytes	x												
Urine protein/creatinine ratio (UPCR)	x	x						x	x	x	x	x	x
Hematology	x	x						x	x	x	x	x	x
PT and PTT	x												
Biochemistry	x	x						x	x	x	x	x	x
Serum creatinine and Cystatin C	x	x		x									
Vitamin D	x												
Serum pregnancy (beta HCG) for females,	x												
Urinalysis - dipstick	x	x						x	x	x	x	x	x
HbsAg, HCV & HIV	x												

Activity Visit Number	**S[4]**	**1**	**2 through 52**	**All odd numbered visits**	**2, 15, 28 and 2 weeks after Visit 53**	**4**	**2, 3, 5, 9, 11**	**7, 20**	**14**	**27**	**33, 47**	**40**	**53**
Short Form Brief Pain Inventory (BPI)	x	x							x	x		x	x
Anti-Drug Antibodies (IgG)[2]	x	x					x	x	x	x	x	x	x
Electrocardiography (ECG)	x	x						x	x	x	x	x	x
Chest X-ray[1]	x												
Quality of Life		x							x	x		x	x
Mainz Severity Score Index (MSSI)		x							x	x		x	x
Request for randomization approval		x											
Randomization		x											
Echocardiography		x								x			x
Cardiac function assessment (stress test)		x								x			x
Cardiac MRI		x								x			x
Brain MRI		X								x			x
Adverse events assessments		x	x										
Mutation analysis		x											
Plasma samples for PK[3]		x			x				x	x			x
Urine lyso Gb3 concentration		x				x		x	x	x		x	x
Plasma Gb3 concentration[2]		x				x		x	x	x		x	x
Plasma Lyso Gb3 concentration[2]		x				x		x	x	x		x	x
Study Drug IV infusion		x	x										x

[1]will be performed only for patients who have not had the test during last 3 months before screening
[2]will be performed pre-infusion
[3]only for 30 enrolled subjects. Time points: pre-infusion (baseline); 0.5 and 1 hour after the beginning of the infusion; at the end of the infusion, at 0.5±0.05, 1±0.25, 2±0.25, 4±0.25, 8±0.25, 24±0.5, 48±3, and 96±3 hours post-infusion and at 14±3 days post-infusion
[4]Re-screening may be performed with Medical Director approval for patients failing to fulfill Inclusion criteria #2 and/or #3 within 2 months of initial screening visit

4. ANALYSIS POPULATIONS (ANALYSIS DATASETS)

4.1 Enrolled Population

The Enrolled population consists of all screened patients and will be used in listings.

4.2 Randomized Population

The Randomized population consists of all randomized patients.

4.3 Intent to Treat Population

The Intent to Treat (ITT) population consists of all randomized patients who received at least one dose (including partial dose) of the study medication (PRX-102 or agalsidase beta) and based on the assigned treatment arm in the randomization.

The ITT is the primary analysis set for all efficacy analysis, and in particular for the primary analysis.

4.4 Per Protocol Population

The Per Protocol (PP) population includes all ITT patients who completed at least 24 months of treatment for the final analysis, with study drug compliance of at least 80%, and with no major protocol violations before DBL which may impact their primary end-point.

The following protocol violations are considered as potential major violations for the purpose of the PP analysis set:

- Patients randomized but not meeting at least one of inclusion criteria 2-5. A patient that did not meet inclusion criterion 7 may be excluded from the PP. Patients who did not meet inclusion criterion 1 (age range) will be discussed on a case-by-case basis and decision will be based on co-morbidities at screening. Similarly, patients who did not meet inclusion criterion 6 (compliance with previous treatment) will be discussed on a case-by-case basis and decision will be based on their longer-term compliance (evaluation of inclusion 6 on a case-by-case basis was added to the SAP after the Blinded Data Review (BDR)).
- Patients randomized but meeting at least one of the exclusion criteria 2-7. A patient who met at least one of the exclusion criteria 1 or 8 – 13 will not be excluded from the analysis set.
- Mis-stratification.
- Patients who did receive the wrong treatment for more than 20% of his/her infusions. Such information may not become available until after DBL.
- Patients who meet discontinuation criteria but not discontinued
- Patients who received prohibited medication
- Patients who had permanent or temporary changes to the dose of Angiotensin converting enzyme (ACEi) / Angiotensin receptor blocker (ARB) will be discussed on a case-by-case basis.

All violations and assignment of patients to the PP set will be reviewed and confirmed at the BDR meetings prior to the DBL and conducted by the blinded team and decisions will be documented. This may include additional major violations that were not anticipated at the time of the SAP. Per IQVIA (CRO responsible for operational aspects of the study) SOP deviations are classified as minor, major and critical following guideline with types of violations. The list of violations includes all violations related to conduct of the study and some may not have impact on the evaluation of the primary end-point. All major and critical violations will be reviewed, and if any deemed to have an impact on the primary end-point, it will be documented and the patient will be excluded from the PP set.

The evaluation of the PP population that was done before the interim analysis and will be repeated in a blinded fashion prior to DBL for the final analyses. Hence, although the same definition is used, the final analysis PP population may be a subset of the interim analysis PP population: patients who had major violations between the DBL of the interim analysis and study completion will be excluded from the PP for the final analysis only. Patients who completed the study prior to the interim will not be re-evaluated.

In a non-inferiority study, the PP and the ITT analysis set should be considered together in the interpretation of the study. In light of that, the PP population will be used for the primary efficacy endpoint as well as for sensitivity and supportive analyses.

4.5 Safety Population

The safety population consists of all patients who are randomized and received any dose (including a partial dose) of the study medication (PRX-102 or agalsidase beta). Assignment is by actual treatment received. The full set of measurements available will be used for the safety analysis All safety analyses will be performed on this population.

5. TREATMENT DESCRIPTIONS

Unless otherwise indicated, in the summary tables, the treatments will be identified by PRX-102 and agalsidase beta.

6. IMPACT OF COVID-19

The impact of COVID-19 on the study was assessed in August 2020 and then revisited as part of the interim analysis Blinded Data Review Meetings (BDRM) in March 2021. As the pandemic is still ongoing, it will be revisited at the BDRM prior to the final analysis and documented in the meeting minutes.

As of March 2021, the impact of COVID-19 is considered to be minimal, as detailed in the list below.

In light of that, for the interim analysis, the Sponsor did not see the need to account for the effect of the pandemic in the primary and safety analyses. Several tables and listings were added (see Section 8.1) in order to be able and discuss the impact and support the choice of no changes on the primary and safety analyses.

Generally speaking, even before the start of the pandemic, the protocol allowed for a mixture of home care and site visits. Home care includes infusion of study drug, blood sampling for the majority of the laboratory tests including primary endpoint and biomarkers, monitoring of Adverse Events (AE), medications and vital signs. Other procedures, including imaging, stress test, echocardiogram and questionnaires are carried out only on site. This use of home care was expanded with no need for protocol amendments and the logistics for this implementation were already in place.

- As of the cut-off date for the interim analysis, no patients have been diagnosed with COVID-19. There were no deaths and no early termination due the pandemic.
- Enrolment for the study was completed in Oct 2019 and hence there is no impact on enrolment.
- The use of home infusions was increased due to COVID-19 restrictions, so there is no or very minimal impact on compliance to study drug.
- Drop out from study was low prior to the pandemic. As of the cut-off date for the interim analysis, one patient has dropped out since the outbreak of the pandemic, but for reasons not related to the pandemic.
- There should be no or very little impact on the primary end-point, since blood samples for serum creatinine are continued to be taken at home (see Section 8.1).
- The protocol allowed for a window of ±3 days in the drug administration visit. The majority of the visits remained within these limits. This is assessed and documented as part of the Blinded Data Review Meeting Report.
- Procedures which must be taken on site may be postponed until the patient can reach the site. Although all efforts were done to perform them even with a delay, in some cases they were cancelled or were conducted as part of the extension study (PB-102-F60) and will be considered missing for the purpose of the analysis of this study. This is assessed and documented as part of the Blinded Data Review Meeting Report.
- For several patients the study was prolonged:
    - For patients who reach Week 104 (visit 53; end of study) and could not perform the end of study visit on site, as planned, the study was extended with the home-care procedures (as detailed above). The patients perform the other procedures of visit 53 when a site visit is possible. In some instances, when the patient performed the delayed visit 53 on site, procedures which required additional coordination of site staff (cardiac MRI, stress test, brain MRI and echocardiogram) were postponed further and performed after the patient switched to the open label extension study PB-102-F60, thus it was decided that those assessments will be excluded from the end of study visit of PB-102-F20 if they performed more than 2 months after the date of Visit 53. This is documented as part of the Blinded Data Review Meeting Report.
    - Some patients were able to perform Week 104 (visit 53) on site, at the planned time, but were not able to switch to the extension study (since the rollover requires site visit), and they continued to be treated at their home with the same drug of study PB-102-F20 until it was possible to have a site visit for the extension study. This is documented as part of the Blinded Data Review Meeting Report.
- The timeline of the interim analysis or its objective are not changed due to COVID-19.

7. STATISTICAL ISSUES

7.1 Statistical Methods

Descriptive statistics, namely number of patients (n), mean and its standard error (SE), standard deviation (SD), median, minimum and maximum for continuous variables, and counts and percentages for categorical variables, will be provided. For some variables, the 25th and 75th percentile will be presented.

Statistical comparison of the efficacy endpoints between treatments of PRX-102 and agalsidase beta will be described in detail in relevant sections of this document.

Unless otherwise specified, summaries by visit will present only scheduled visits based on the protocol planned visits for each procedure. Procedures which were performed in an unscheduled visit or in scheduled visits in which the procedure was not planned per protocol, will only be listed. Data collected in premature withdrawal visit will be listed but not tabulated.

Listings will show data collected on all enrolled subjects (including screening failures), when applicable. In listings: patients will be sorted to show treated patients first (PRX-102 followed by agalsidase beta) and then patients who were screening failures. Visits (scheduled and unscheduled) within subject will be shown chronologically based on the date of the visit.

Due to the COVID-19 pandemic, the study may be prolonged beyond Week 104 (visit 53 data collected beyond visit 53 will be listed and may be included in the analysis (discussed for each analysis, as relevant). These data will not be presented in summary tables by visit.

7.2 Missing Data

Imputation of missing data associated with AE severity and relatedness are described in Section 11.2.

Several imputation strategies will be used to impute missing data as part of a sensitivity analysis supporting the primary analyses (see Section 9.4).

Below are imputation rules related to missing or incomplete dates of medication or Acute Kidney Injury AE.

7.2.1 Partial or Missing Dates of Medication

The following imputations will be used in case of incomplete dates of agalsidase-beta treatment required for the calculation of its duration prior to the study:

- The middle of the month (i.e., 15th) will be used if only the day is missing.
- In case the year is not missing, but the month is missing, the month will be imputed to July. If the day is missing as well then it will be imputed to the 1st of July
- No imputation will be done in case the year is missing.

No imputation will be done in case of fully missing medication date.

In some of the analyses, a flag of whether a medication was used at a certain visit is needed (e.g., use of Angiotensin-Converting Enzyme Inhibitor (ACEi) or Angiotensin Receptor Blocker (ARB) treatment at baseline or usage of pain medication at baseline or in the last visit). In case that partial start date and/or end date exist, imputation will be based on the above rules and determination of the flag will be based on the imputed dates and comparison with the date of the visit of interest.

In case that the year is missing from the start date, the determination of usage of a medication at different visits will be based on the stop date of the medication. If the year of the stop date is missing, then the determination will be based on the "Ongoing" status of the medication form as follows: if the ongoing status is Yes then it is assumed that the medication was taken throughout the study (including at baseline and last visits), and if the ongoing status is No then it is assumed the medication was stopped prior to the baseline visit.

Since information on infusion premedication is collected at both the medication form and the drug administration form then determination of usage of infusion premedication at a given visit is based on a different algorithm described in Section 11.8.2.

7.2.2 Partial or Missing Dates of Episodes of Acute Kidney Injury

Episodes of Acute Kidney Injury (AKI) are reported by the investigators as an AE. An AKI episode is defined between the start and the end date of the AKI AE. The following rules will be followed in the case of an incomplete start or end date of an AKI event:

- AKI start date
    - If month and year are available and day is missing, the 1st of the month will be imputed. In case the event is during the month of the first infusion, it will be imputed to the date of 1st infusion.
    - If month is missing and year is available then if the year is the same as the year of 1st infusion date, then should be the date of 1st infusion. Otherwise, the date should be set to January 1st.
    - If year is missing then the date should be imputed to date of 1st infusion.
- AKI end date
    - If month and year are available and day is missing, the last day of the month will be imputed. In case the event is during the month of the last infusion, it will be imputed to the date of end of study.
    - If month is missing and year is available then if the year is the same as the year of last infusion the date should be the last day in the study. Otherwise, the date should be set to December 31st.
    - If year is missing then the date should be imputed to last day in the study.

Serum creatinine samples taken for the study while the AKI AE was still considered as on-going will be excluded from the eGFR slope calculation and summary tables and considered to be missing. See Section 9.1 for more details.

7.3 Baseline Definition

The baseline for this study is defined as the last assessment prior to the first treatment infusion. The exception to this rule is cardiac MRI. Due to a different scheduling of cardiac MRI and, in some cases, repeated cardiac MRI due to inability to interpret it, delayed baseline cardiac MRI, with a delay of up to 60 days will be considered as baseline (Perry et al, 2019).

7.4 Subgroup Analysis

Subgroup analyses will be conducted based on baseline characteristics and demographics for selected efficacy and safety endpoints and only if the size of each of the groups is at least 10 (combined over the two treatment arms). If a patient is missing a value at baseline for one or more of the subgroups, the classification will be based on the value at screening.

Whether a subgroup analysis will be conducted for a specific endpoint, and if yes for which of the subgroups the analysis will be done, will be discussed in the analysis section of the specific endpoint. The selection of subgroup will be from the following list:

- Gender (Male or Female)

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

- FD classification (Classic/Non-Classic). In order to be classified as FD classic, a patient should have ≤ 5% mean of lab normal ranges residual enzymatic activity in plasma or leukocytes at baseline visit and at least one Fabry specific symptom: Cornea Verticillata, Acroparesthesias, Angiokeratomas. Evaluation of Fabry specific sympom is based on the Fabry Disease Medical History collected at screening visit. Mean normal range is defined as (lower limit of normal + upper limit of normal)/2) and % of mean of lab normal range is defined as (100 × test result / mean normal range); this was calculated within the EDC system and will not be re-derived for the purpose of the analysis. In case of missing information for the symptoms or residual activity, which does not allow for clear classification as classic or non-classic, the FD classification will be missing and the subject will be excluded from the sub-group analysis (for example, if a patient meets the symptoms requirement and have a plasma residual activity of 10% but the leukocytes residual activity is missing then the classification will be missing and the patient will be excluded from the subgroup analysis. As another example, if a patient meets the symptoms requirement and have a plasma residual activity of 3% and the leukocytes residual activity is missing, it is still possible to classify the patient as Classic and the patient will be included in the subgroup analysis).
- Baseline eGFR (≤ 60; 60 < and ≤ 90; > 90 mL/min/1.73m^2)

- Annualized slope of eGFR (≤ -5; > -5 mL/min/1.73m^2/year)

- Use of ACEi or ARB treatment at baseline (Yes/No). The usage of ACEi or ARB is based on classification in the medication form in the eCRF. Based on this form, it is possible to identify

patients who received ACEi or ARB at Baseline date. All other patients will be classified as "No" for this subgroup.

- Region (United States (US)/ex-US)
- UPCR categories (≤ 0.5 gr/gr; 0.5 < and < 1 gr/gr; ≥1 gr/gr).

7.5 Interim Analysis and Unblinding

The interim analysis was conducted after the last patient randomized completed 12 months of treatment. The primary objective was to demonstrate the non-inferiority of PRX-102 compared to agalsidase beta. The interim analysis was used for submitting an MAA to the EMA, so all other efficacy and safety analyses have been performed at this point and CSR has been written.

The objective of the interim analysis was to support submission of the MAA, with only some individuals at the Sponsor being unblinded at this point to treatment assignment. To protect the blinding of the final analysis, an unblinded data access plan describing the measures to be taken to ensure the blinding of the interim study results and patients' treatment assignments is discussed in Appendix A.1 of this SAP (Section 16). Due to the additional discussions and agreements with the FDA following the full approval of agalsidase beta, some changes had to made to this plan.

7.5.1 Merging Randomization Codes with Clinical Data

Study Data Tabulation Model (SDTM) programming is performed by the Data Management vendor of the study who remains blinded until final analysis, and hence were not able to merge the randomization codes with the clinical data as part of the interim analysis SDTM.

For the interim analysis, merging of the randomization codes was performed by the unblinded team of the vendor that provides statistical analysis services. For consistency the same process will be followed for the final analysis.

7.6 Multiplicity Considerations

7.6.1 Primary Endpoints – Interim and Final Analyses

The primary efficacy endpoint for both interim and final analyses is the mean annualized change (slope) in estimated glomerular filtration rate (eGFR). Both evaluate non-inferiority analysis and the assessment is done using a one-sided alpha level of 0.025.

For regulatory purposes, demonstration of non-inferiority of PRX-102 compared to agalsidase beta at 12 months for submission of MAA to the European Medicines Agency and non-inferiority at 24 months for FDA BLA submission will be considered trial success.

Since there is no intention to stop the study for futility or efficacy and no plan for study adaptations based on outcomes of the interim analysis, no alpha penalty for the final superiority analysis is needed.

7.6.2 Other Efficacy Endpoints

No multiplicity adjustment will be made to other efficacy endpoints or other time-points, sensitivity/supportive analyses, and sub-group analyses. In all these cases, nominal significance will be reported but no claims for significance will be made.

8. DEMOGRAPHICS AND STUDY SUMMARY

8.1 Patient Disposition

The number and percentage of patients who were screened, randomized, completed 12 months of treatment (i.e.: subjects who reached the 12 months study milestone, completed 24 months (i.e.: subjects who have completed the study as per protocol), and discontinued will be summarized by treatment group and overall. The documented reasons for screen failures will be tabulated. The number and percentage of patients who discontinued will also be summarized for each reason of the discontinuation. The number of patients with study prolongation due to COVID-19 will be summarized. In addition, patients who discontinued will be classified based on their time of discontinuation (before or after the interim analysis) and the number and percentage of patients in each of these two categories will be presented.

The number of patients in each of the analysis sets (ITT, Safety and PP) will be summarized by treatment group and overall. For each patient, the reason for exclusion from analysis population will be detailed in listing. The reasons for exclusion from the PP set will be summarized by treatment arm.

Protocol deviations were classified in a blinded manner as minor, major and critical (critical are considered more severe than major and are reserved mainly for Good Clinical Practice violations). A summary table will show the number of subjects with at least one major or critical deviations as well as with breakdown by violation type. All protocol deviations will be listed. The listing will include also violations related to COVID-19 (e.g., visits out of windows).

Time to discontinuation will be presented graphically by Kaplan-Meier (KM), where time will be from 1st infusion. For subjects who completed the study, the time should be censored at the date of last infusion.

To assess the impact of COVID-19, the compliance with infusions and blood collection for eGFR will be summarized descriptively and listed. The compliance will be calculated by the number of actual infusions/blood samples divided by the number of planned infusions/blood samples after March 1st, 2020. The analysis will be conducted only for patients who were still ongoing as of March 1st, 2020.

8.2 Demographics

The demographics (age (calculated using intck function in SAS), gender, race, and ethnicity) will be summarized using descriptive statistics by treatment group, overall, and for each of the subgroups in Section 7.4. This analysis will be done on the ITT set.

8.3 Baseline Characteristics

The following baseline characteristics will be summarized on the ITT analysis set by descriptive statistics by treatment group, overall and for each of the subgroups in Section 7.4:

- Weight
- Height
- Region (US, ex-US)
- Duration of the last continuous agalsidase-beta treatment (months). Last treatment refers to patients who had several periods of treatment with agalsidase-beta in the past. In case of several records with a gap ≤ 14 days between the end date and the start date of the following record they are considered as same treatment. The end-date of agalsidase-beta should be taken as the F20 treatment start date (regardless of whether there is an end-date for agalsidase-beta in the database).
- % Residual enzyme activity in leukocyte (defined as the value in leukocyte × 100/83.5, where 83.5 nmol/hr/mg protein is the mean of reference range)
- % Residual enzyme activity in plasma (defined as the value in plasma ×100/12.95, where 12.95 nmol/ hr/mL is the mean of reference range)
- eGFR (estimated using CKD-EPI equation and expressed in ml/min/1.73 m^2); See Section 9.1.
- eGFR slope (ml/min/1.73 m^2/year) at screening (based on historical serum creatinine and screening serum creatinine measures) as calculated within the EDC system (using linear regression).
- eGFR slope (ml/min/1.73 m^2/year) at baseline (based on historical serum creatinine, screening and baseline serum creatinine measures as well as any unscheduled eGFR assessments before baseline visit) and calculated using simple linear regression in a similar way to the approach described in Section 9.3.3.
- Baseline eGFR class (≤ 60; 60 < and ≤ 90; > 90 mL/min/1.73m^2)
- Baseline eGFR slope class (⩽ -5; > -5 mL/min/1.73m^2/year)
- Plasma Lyso-Gb3
- Fabry disease (FD) classification (classic / non-classic) (See Section 7.4 for definition. In case of missing data subjects will not be classified)
- UPCR Categories (≤ 0.5 gr/gr; 0.5 < and < 1 gr/gr; ≥1 gr/gr)
- UPCR Stratification (based on screening values) (< 1gr/gr; ≥ 1 gr/gr)
- Treatment with ACEi or ARB (yes/no)
- Premedication use for agalsidase-beta infusion prior to 1st infusion (yes / no)
- ADA status for PRX-102 (positive /negative). The determination of the status is based on the results of the IgG for PRX-102 at baseline (Section 11.7).
- ADA status for agalsidase-beta (positive /negative). The determination of the status is based on the results of the IgG at baseline for agalsidase-beta (Section 11.7).

8.4 Fabry Disease Medical History

Fabry disease medical history by body system and conditions (as collected in the Electronic Case Report Form (eCRF)) will be tabulated by treatment arm and for each of the subgroups in Section 7.4. The analysis will be done on the ITT analysis set.

Information on Fabry disease medical history is collected from 4 different forms within the eCRF: Fabry Disease Medical History, Fabry Disease Diagnosis Per Protocol, Fabry Disease Past Treatment and Mutations Analysis. Data collected on these forms will be listed.

Age of Fabry disease diagnosis will be calculated using intck function in SAS and will be included in the relative listing.

8.5 Other Medical History

Other medical history will be summarized by System Organ Class (SOC) and Preferred Terms (PT) by treatment group and for each of the subgroups in Section 7.4. The analysis will be done on the ITT analysis set. SOC will be sorted alphabetically: within SOC, PT will be sorted in a decreasing order of PRX-102 frequency.

8.6 Treatment Compliance

Treatment compliance will be assessed by the percentage of the number of infusions (partial or complete) out of the expected number of infusions for each patient based on the patient treatment duration. For patients who terminated early, the expected number of infusions will be based on their treatment start date and date of discontinuation. This will be done regardless of reason of discontinuation. Treatment compliance will be summarized by treatment group using the ITT set. For patients whose study was prolonged due to COVID-19, the compliance will be based on their extended time in the study.

Compliance will be summarized also by the following categories: $< 60\%$; $60\% \leq$ and $< 80\%$; $\geq 80\%$.

9. ANALYSIS OF PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the mean annualized change (slope) in estimated glomerular filtration rate (eGFR). The eGFR is not measured directly but is derived from the value of the serum creatinine and from patient characteristics, with close to 30 planned visits over 2 years in which the serum creatinine is evaluated.

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

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

For the interim analysis the models considered were the RIRS, RI and two-stage with ANCOVA. The two-stage with quantile regression was added following a recent publication by Ortiz et al (2021) in which the median eGFR slope of Fabrazyme was compared to the median eGFR slope of un-treated Fabry patients. Unlike linear regression which makes distributional assumptions (i.e., normality), the quantile regression makes no such assumptions, and is robust against outliers (Koenker and Bassett (1978; 1982); Hao and Naiman, D. (2007)).

Following the Ortiz publication, extensive simulations were performed and shared with the FDA, and it was agreed to use the two-stage with quantile regression on the ITT set as the primary model for the final analysis. For non-inferiority, both the ITT and PP should be considered when interpreting the study, so the analysis will be performed also on the PP set.

The two-stage with ANCOVA, RI, RIRS on the ITT and PP will be considered as supportive analyses as they deal with a different estimand.

For all of the analyses discussed in this section, time will be measured relative to day of 1st infusion.

This section is laid out as follows: Section 9.1 discusses the derivation of the eGFR from serum creatinine; Section 9.2 provides technical details for the proposed two-stage with quantile regression model; Section 9.3 describes the planned sensitivity and supportive analysis, Section 9.4 describes the sensitivity analyses to account for missing data, Section 9.5 describes the sensitivity analysis for AKI episodes and Section 9.6 describes the planned sub-groups to be examined for the primary endpoint.

All analysis described in this section will be conducted on the ITT analysis set. In addition, some of the analysis will be conducted also on the PP analysis set as sensitivity analysis

9.1 eGFR Derivation

eGFR will be derived based on the value of the serum creatinine using the CKD-EPI formula and rounded to 2 decimal places:

$$\text{eGFR (mL/min/1.73 m}^2) = 141 \times \min(\text{Scr}/\kappa,1)^{\alpha} \times \max(\text{Scr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018 \text{ [if female]} \times 1.159 \text{ [if black / African American]},$$

where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1. Age is the actual age when the patient's serum creatinine is collected.

Results of the serum creatinine test at the screening visit appear twice in the data-base: once as part of the data transferred from the central laboratory and once in the eCRF (once the site receives from the central laboratory, the value is entered into the eCRF to check the eligibility in terms of eGFR value and the eGFR slope). These two values should be identical. In the listings, by-visit tables of eGFR and slope calculations, the value entered into eCRF should be used anyway.

In case of two identical serum creatinine measurements taken at same date and time but assigned to two different visits, then it should be used only once and assigned to the visit that was performed on that date.

AKI Episodes are reported by the investigators as an AE. Serum creatinine measurements, if any, during an AKI episode will be excluded from the eGFR summary tables and any analysis related to eGFR. An AKI episode is defined between the start and the end date of the AKI AE. The number of eGFR measurements which are excluded from the analysis will be summarized by treatment arm.

eGFR values associated with the AKI events will only be listed (and flagged to identify that they were excluded from tables).

The eGFR values and the change from Baseline in these values will be summarized by visit and treatment arm for the ITT and the PP set.

In case that serum creatinine value (and hence eGFR) is missing in a planned visit, but the test was performed up to 17 days after the date of the planned visit, the serum creatinine and eGFR will be mapped to the planned visit and will be included in the summary tables by visit.

Mean eGFR values and change from baseline over time will be plotted by treatment group as well as a graph of eGFR boxplots over time.

9.2 Primary Efficacy Analysis – Two Stage with Quantile Regression

This analysis will be performed on the ITT and the PP analysis sets.

With this approach, at the first stage, the individual annualized mean change (slope) in eGFR will be estimated for each patient using the following linear regression model:

$$eGFR = \alpha + \beta \times [\text{time in year}].$$

The slope β (mL/min/1.73 m^2 / year) will be an estimate of the individual patient's annualized mean change in eGFR. The "[time in year]" in the formula denotes time measured in years, from Baseline (defined for the purpose of the calculation as date of 1st infusion) to the respective visit, and will be estimated by (date of the visit – date of Baseline)/365.25. All data points for a patient will be used to estimate the slope, excluding any eGFR values measured during an AKI episode, which will be considered missing. For patients whose study was prolonged in light of COVID-19, the additional eGFR measurements will be included in the analysis. The linear model will be fit for all patients with at least 4 eGFR measurements (after exclusion of eGFR during AKI). For patients with fewer than 4 measurements, the slope will be missing. Considering the low rate of early termination and high compliance with visits, it is expected that the number of patients with missing slopes will be very low. The individual slopes obtained at the 1st stage of the analysis will be summarized descriptively and individual slopes will be listed.

At the 2nd stage, the annualized mean change (slope) of the eGFR between the two treatment arms will be compared using quantile regression estimating the median slopes. The dependent variable will be the slope of each individual patient and the model will include the following covariates:

- Intercept
- Treatment arm (PRX-102; agalsidase beta). This is the main parameter of interest.

The estimated median slopes for the two treatment groups and their difference, and the corresponding 95% CI obtained from the quantile regression will be presented. The quantile regression analysis will be implemented using SAS PROC QUANTREG with the code and detailed description provided in Appendix A.2 (Section 17).

Non-inferiority will be declared if the lower bound of the confidence interval for the treatment difference (PRX-102 minus agalsidase beta) is greater or equal to -3.0 mL/min/1.73 m^2/year.

9.3 Sensitivity and Supportive Analyses for the Primary Analysis

Several sensitivity and supportive analyses will be conducted. The sensitivity analysis will deal with the same estimand as the primary analysis, and will differ in the covariates used. The supportive analysis will deal with a different estimands (mean using RIRS, RI, and two-stage with ANCOVA; mean eGFR change from baseline using a Mixed Model Repeated Measure (MMRM) and other quantiles using quantile regression. Additional sensitivity analysis may be conducted as post-hoc. In such case, they will be reported as post-hoc in the CSR.

A forest plot will be created that includes the results of the primary analysis as well as sensitivity and supportive analyses described in this section and in Section 9.4.

All the analyses described in this section will be conducted on the ITT and PP sets.

9.3.1 Sensitivity Analysis – Two Stage with Quantile Regression – Different Covariates

The two-stage with quantile regression will be repeated, where in addition to the intercept and treatment arm, the model will include the stratification factor ((UPCR < 1; ≥ 1 gr/gr).

The estimated median slopes for the two treatment groups and their difference, and the corresponding 95% CI obtained from the quantile regression will be presented. The estimated median for the two groups will be done for the balanced case as described in Appendix A.2 (Section 17).

Non-inferiority will be declared if the lower bound of the confidence interval for the treatment difference (PRX-102 minus agalsidase beta) is greater or equal to -3.0 mL/min/1.73 m^2/year.

9.3.2 Supportive Analysis – Random Intercept Random Slope Longitudinal Model

The analysis compares the annualized mean change (slope) of the estimated glomerular filtration rate (eGFR) between the PRX-102 group and the agalsidase beta (Control) group. A random intercept random slope longitudinal mixed model will be used to compare the eGFR slopes of the two groups.

The general form of the mixed model is,

$$Y = X\beta + Zb + \varepsilon$$

where Y denotes the eGFR values at the different time points. The design matrix, X, represents the fixed part of the model and will include the following terms:

- Intercept
- Treatment arm (PRX-102; agalsidase beta)
- Time (actual time of measurements (in years) considered as a continuous variable)

- Treatment arm by time interaction. This is the main parameter of interest.

The random effect part, Z, will include a random intercept and a random slope and an unstructured covariance matrix (dimension 2 X 2) will be used. The covariance of the error term, ε, is assumed to be a diagonal matrix with equal variances along the diagonal. The restricted maximum likelihood approach will be used to estimate the variance components, and the Kenward-Roger approximation will be used to estimate the denominator degrees of freedom.

For patients whose study was prolonged in light of COVID-19, the additional eGFR measurements will be included in the analysis. All eGFR values collected during an AKI episode will be set to missing. Missing data (due to AKI or other reasons) will not be imputed.

The estimated slopes for each of the two treatment groups will be obtained from the mixed model and the estimated difference in slopes is the coefficient associated with the time by treatment interaction term. The corresponding 95% confidence intervals (CI) for all three estimates will be presented. The analysis will be implemented using SAS PROC MIXED with the code and detailed description provided in Appendix A.2 (Section 17).

Non-inferiority will be declared if the lower bound of the 95% CI for the interaction term of treatment group by time is greater or equal to -3.0 mL/min/1.73 m^2/year, the prespecified non-inferiority margin.

9.3.3 Supportive Analysis: Two-Stage Approach Using Analysis of Covariance

The 1st stage of this approach is identical to the 1st stage of the two-stage with quantile regression.

At the next stage, the annualized mean change (slope) of the eGFR between the two treatment arms will be compared using Analysis of Covariance (ANCOVA). The dependent variable will be the slope of each individual patient and the model will include the following covariates:

- Intercept

- Treatment arm (PRX-102; agalsidase beta). This is the main parameter of interest.

The estimated means of the slopes for the two treatment groups and their difference, and the corresponding 95% CI obtained from the ANCOVA model will be presented. The ANCOVA analysis will be implemented using SAS PROC MIXED with the code and detailed description provided in Appendix A.2 (Section 17).

Non-inferiority will be declared if the lower bound of the confidence interval for the treatment difference (PRX-102 minus agalsidase beta) is greater or equal to -3.0 mL/min/1.73 m^2/year.

9.3.4 Supportive Analysis: Random Intercept (RI) Longitudinal Model

The model is similar to the model described in Section 9.3.2, but here the random effect, Z, will include only one random component, namely the intercept. The correlation between measurements within a patient is due to the common random intercept. This implies that the overall covariance matrix of Y is a block diagonal with a compound symmetry structure within patients. A robust estimate of the covariance matrix will be computed using a sandwich estimator with the

EMPIRICAL option in the PROC MIXED statement. Note that this option does not allow for usage of the Kenward-Roger degrees of freedom. The analysis will be implemented using SAS PROC MIXED with the code and detailed description provided in Section 17.2. Evaluation of non-inferiority and superiority will be done in the same manner as for the RIRS model.

9.3.5 Supportive Analysis: eGFR Change from Baseline

As an additional supportive analysis, the MMRM with the change from Baseline in eGFR will be fit. In this model the response variable will be the change from Baseline in eGFR at each scheduled study visit where eGFR is measured. eGFR values that were mapped to a visit with missing eGFR (Section 9.1) will be included in the analysis.

The model will include the following covariates:

- Intercept
- Baseline eGFR value
- Visit (as a class variable)
- Treatment arm (PRX-102; agalsidase beta)
- Visit by treatment arm group interaction

The first choice for the within-patient correlations will be an unstructured covariance matrix. It is important to note that due to the large number of visits, this matrix will be of dimension close to 30×30 and the model may not converge. If the model does not converge, the model will be fitted assuming a heterogeneous Toeplitz structure, followed by a homogeneous Toeplitz structure and then a compound symmetry structure until a stable model is achieved. Only data at scheduled visits will be used for this analysis. The Kenward-Roger degrees of freedom approximation will be used in calculating the Least Squares Means (LSMEANS) for the difference in the change from Baseline at 1 year and at 2 years between the two treatment arms.

The pseudo-SAS code for this analysis can be found in Appendix A.2 (Section 17).

Non-inferiority will be declared if the lower bound of the 95% CI for the contrast between treatment arms at the 2-year visit (Week 104) is greater than or equal to -6.0 mL/min/1.73 m^2/year.

9.3.6 Supportive Analysis: Two-stage with Quantile Regression – Additional Quantiles

The two-stage with quantile regression will be repeated for the ITT and PP, where in the 2nd stage the 25th and 75th quantiles as well as their difference will be estimated instead of the median.

9.4 Sensitivity Analysis for Missing Data

Analyses described in this section will be performed on the ITT set.

The primary analysis (Section 9.2) and the sensitivity analysis (Section 9.3) assume that the data are Missing At Random (MAR). The Sponsor acknowledges that missing data are a potential source of bias when analyzing data from clinical trials, particularly if the proportion of missing

values is substantial. Yet, a low rate of early termination has occurred in this 2-year study. The rate of intermediate missing eGFR data is low as well (out of the 30 planned eGFR assessments during the study, including Baseline, a small percent is missing). In light of this, the impact of missing data on the results appears to be minimal and the assumption of MAR seems reasonable. The impact of missing data will be assessed in sensitivity analyses, as outlined below.

The Sponsor made an effort to minimize the potential impact of COVID-19, particularly on the collection of blood for serum creatinine evaluation. There was one case of early termination since the start of the pandemic for a reason not related to the pandemic, and at the time of interim analysis there was minimal impact on the intermediate serum creatinine collection (this will be assessed by the blinded team as part of the blinded data review meeting).

The number and percent of early terminations will be summarized by treatment arms including the reasons for withdrawal. Time to discontinuation by treatment will be presented using a Kaplan-Meier analysis. In addition, the number of AKI episodes that precluded eGFR calculations and the number of patients with these episodes will be summarized by treatment arm.

In addition, Multiple Imputation (MI) will be used to assess the impact of missing data, as described below.

MI under the MAR assumption will be conducted for patients who early terminated. Missing data will be imputed within each treatment arm. Details are provided in Appendix A.2 (Section 17.6).

9.5 Sensitivity Analysis for Elevated Serum Creatinine

The analysis described in this section will be conducted on the ITT set.

AKI Episodes are reported by the investigators as an AE and eGFR measurements during the time of the AE will be excluded from the analysis (see Section 9.1).

As a sensitivity analysis to the primary analysis, in addition to the excluded eGFR values during an AKI episode, any eGFR value associated with events of an elevated serum creatinine, as described in the study protocol, will be excluded from the analysis as well. An elevated serum creatinine is defined by a 1.5-fold increase or greater compared to the immediate previous serum creatinine value as long as that measurement was taken no more than 34 days before (visits are every two weeks, so this allows for a situation that previous visit is missing and 6 days to account for allowed window for the visits).

The number of such observations and the number of patients with at least one such an observation will be summarized by treatment arm.

9.6 Subgroups

The analyses described in this section will be conducted on the ITT set.

Descriptive statistics of eGFR values and the change from Baseline will be summarized by visit and treatment arm for all subgroups defined in Section 7.4.

The statistical approach used for the primary analysis (Section 9.2) will be repeated for all these sub-groups.

For each of the subgroups, the primary model (i.e., two-stage with quantile regression) will be used for each level of the subgroup separately with the same covariates as the primary model. A 95% CI for the treatment effect within each level of a subgroup will be presented.

The results will be presented graphically by a forest plot.

10. ANALYSIS OF SECONDARY EFFICACY ENDPOINTS

All the analyses of secondary end-points will be performed on the ITT set.

10.1 Analysis of Fabry Kidney Disease Therapeutic Goals

Patients will be classified into three kidney disease (KD) groups, based on their eGFR slope at baseline. The therapeutic goals (See Wanner et al., 2018) will be evaluated for each of the categories.

Table 2: Therapeutic Goals

KD Group	Criteria	Therapeutic Goals
Stable KD	Slope at baseline ≥ -3 mL/min/1.73 m^2/year	post-treatment slope ≥ -3 mL/min/1.73 m^2/year
Progressing KD	Slope at baseline ≥ -5 and < -3 mL/min/1.73 m^2/year	post-treatment slope ≥ -3 mL/min/1.73 m^2/year
Fast-progressing KD	Slope at baseline< -5 mL/min/1.73 m^2/year	post-treatment slope ≥ -5 mL/min/1.73m^2/year, OR > 50% decrease in progression (i.e., (eGFR slope at baseline – post-treatment eGFR slope) / eGFR slope at baseline > 50%)

The individual slopes calculated in the 1st stage described in Section 9.2 will be used for the analyses described in this section.

The number and percentage of patients achieving therapeutic goals (yes/no) will be presented overall and for the three KD groups by treatment arm. A 95% Clopper Pearson CI for the difference in the percentages between the two treatment groups will be presented. The analysis will be repeated for sub-groups: gender, Fabry disease status, ADA status and UPCR category at Baseline.

A shift table between the three categories from Baseline to post-treatment will be presented by treatment arm.

The analysis will be repeated for the Per-Protocol set.

10.2 Plasma Lyso-Gb3 Concentrations

Descriptive statistics of plasma Lyso-Gb3 concentration (nM) will be summarized at each visit. The change and percent change from Baseline will be summarized as well.

A MMRM approach will be used to model the change from baseline of log of plasma Lyso-Gb3. The outcome will consist of the change from baseline of log of Lyso-Gb3 at each post baseline visit. The variables to be included in the model are log plasma Lyso-Gb3 at Baseline, visit (class variable), treatment (class variable), visit by treatment interaction term.

The first choice for the within-patient correlations will be an unstructured covariance structure. If the model does not converge, the model will be fitted assuming a heterogeneous Toeplitz structure, followed by a homogeneous Toeplitz structure, and then a compound symmetry structure until a stable model is achieved. Only data at scheduled visits will be used for this analysis. Missing data will not be imputed. The Kenward-Roger degrees of freedom approximation will be used. Appendix A.2 (Section 17) provides details and pseudo-SAS code for this analysis.

95% CI for the contrast between treatment arms at 1-year visit (Week 52) and at the 2-year visit (Week 104) will be presented, as well as the two-sided p-value for the null hypothesis of no difference between the two arms. Adjusted LSMEANS of the two treatment arms for the change from Baseline to 1-year and from Baseline to 2-year will be presented along with a 95% CI.

The analysis will be repeated for the following subgroups: ADA status, gender and FD classification. A 95% CI for the treatment effect within each level of a subgroup will be presented.

The mean ± SE of plasma Lyso-Gb3 and the change from baseline over time will be presented graphically by treatment group and by gender.

The number and percentage of patients who improved by at least 30%, 40% and 50% will be summarized at Week 52 and at Week 104 by treatment arm and for the above sub-groups.

10.3 Plasma Gb3 and Urine Lyso-Gb3 Concentrations

Descriptive statistics of plasma Gb3 concentration (nM), and urine Lyso-Gb3 concentration (pM/mM Creatinine) will be summarized at each visit by treatment group. The change and percent change from Baseline will be summarized as well. A 95% CI for the difference between the two arms will be presented for the change from baseline and percent change from baseline using t-distribution for 2-samples.

The analysis will be repeated by gender and by ADA status.

10.4 Cardiac Assessments

10.4.1 Cardiac MRI

Left ventricular mass (LVM), left ventricular mass index (LVMI, indexed to patient's body surface area (g/m^2)), and left ventricular ejection fraction (%EF) will be summarized using descriptive statistics for each visit that includes these evaluations. The change from baseline will be summarized. A 95% CI for the difference between the two in the change from baseline to Week 52 and to Week 104 arms will be presented using t-distribution for 2-samples. The LVM and LVMI analysis will be done separately for patients who have hypertrophy at baseline, patients who don't have this abnormality, and patients whose hypertrophy at baseline is missing. Hypertrophy is defined as LVMI above 91 g/m^2 for males and LVMI above 77 g/m^2 for female (Kawel-Boehm (2015)).

In case that LVM at a visit is available, and LVMi is missing, the following rule will be used to calculate LVMi:

$$LVMI = LVM / BSA.$$

Du Bois and Du Bois (1916) will be used to calculate the BSA:

$$BSA = 0.007184 \times W^{0.425} \times H^{0.725},$$

where H is the height (cm) measured at screening and W is the weight (kg) measured at the same visit of the cardiac MRI. In case that weight at the visit is missing, the last available weight prior to the visit will be used. This is the formula which is used by the vendor that provides the cardiac MRI assessments.

The analysis will be repeated by gender and by FD classification.

A shift from baseline to Week 52 and to Week 104 in the number of left ventricular fibrosis segments (based on cardiac MRI) will be presented in a shift table by treatment group.

A shift from baseline to Week 52 and to Week 104 in the number of patients who have Hypertrophy at Baseline (Yes / No / Missing) will be presented by treatment group and by treatment group and gender.

For definition of baseline for cardiac MRI, see Section 7.3. For the same reasons discussed in Section 7.3, delays of up to 60 days in the performance of cardiac MRI at Week 52 or Week 104 will be considered with planned visit.

Not all patients can perform cardiac MRI (e.g., patients with pacemaker), and for others only part of the assessments can be done (e.g., patients who cannot receive contrast dye to avoid the contrast nephrotoxicity). This information will be included in the listing.

For some patients, cardiac MRI at Week 104 was postponed due to COVID-19 and eventually performed as part of the extension study, so the data will not be analyzed as part of this study (Section 6).

10.4.2 Echocardiogram

Number and percentages of patients with respect to echocardiogram qualitative assessments results (normal / other) regarding Aortic, Mitral, Tricuspid, and Pulmonic will be presented in a shift table from baseline to Week 52 and Week 104 by treatment group.

LVM calculated by echocardiogram will only be listed.

For some patients, echocardiogram at Week 104 was postpone due to COVID-19 and eventually performed as part of the extension study, so the data will not be analyzed as part of this study (Section 6).

10.5 Short Form Brief Pain Inventory (BPI)

Descriptive statistics of the qualitative assessments and change from baseline regarding pain severity (worst pain in the last 24 hours, least pain in the last 24 hours, right now and average) and pain interference will be summarized at each visit by treatment group. For each of the pain

severities and pain interferences assessments, a 95% CI based on the t-distribution for a paired sample, for the change from baseline, will be presented. A 95% CI of the change from Baseline to 12 months and to 24 months between the two treatment groups will be done using t-distribution for two samples.

The analysis for the average pain over the last 24 hours will also be done for the following subgroups: ADA status, gender and FD classification.

The proportion of patients whose average pain severity did not change or improved compared to baseline (the difference from baseline is ≤ 0) and the proportion of patients whose average pain severity deteriorated compared to baseline (the difference from baseline > 0) will be summarized at each visit by treatment group. If the BPI assessment was postponed due to COVID-19, the analysis will use the data collected on site at the first time a patient could attend an on-site visit.

10.6 Urine Protein/Creatinine Ratio

Urine protein/creatinine ratio (UPCR), by a spot urine test, will be summarized at each visit by treatment group using the following three categories:

- UPCR ≤ 0.5 gr/gr,
- 0.5 < UPCR < 1gr/gr
- 1≤ UPCR gr/gr

The laboratory limit of detection for urine protein is 4 mg/dL, and for a large number of the measurements, the protein is undetectable ('< 4 mg/dL'), resulting in a UPCR of '< x gr/gr' (where x is calculated by 4 divided by the measured level of creatine and the '<'comes from the protein value). Any such observation will be classified in one of the above categories, ignoring the '<' sign in the observation. This is considered a conservative assignment to categories.

The analysis will be repeated for the following sub-groups: ADA status, gender, use of ACEi/ARB and FD classification.

The shift from baseline to Week 52 (or early termination) and to Week 104 (or early termination), between UPCR categories, will be presented by treatment group. The tables will show the number and percentage of patients in each cell.

The UPCR listing will include the actual observation (i.e., '<' sign will not be removed) as well as the assigned category.

10.7 Stress Test

Qualitative evaluation (yes/no) of symptoms (chest pain, shortness of breath, dizziness, palpitations, and other) and the overall impression: normal stress test (yes/no) will be summarized by count and percentage at each visit by treatment group.

In addition, for overall impression only, a shift from baseline will be presented: normal stress test (yes / no). For patients who terminated early, the table will show their shift to their last assessment prior to discontinuation.

Stress test data ay Week 104 may be missing for a few patients due to COVID-19 (Section 6).

10.8 Mainz Severity Score Index (MSSI)

Descriptive statistics of the total scores and change from baseline of each of the domains (general, neurological, cardiovascular, renal dysfunction, and overall score (sum of these four scores)) will be summarized at each visit the MSSI is evaluated by treatment group. A 95% CI, based on a t-distribution for a paired sample, for the change from baseline, within each treatment arm, will be presented. A 95% CI based on a t-distribution for two samples in the change from baseline to Week 52 and to Week 104 will be presented.

A total MSSI score < 20 is considered as mild; a score $20 \leq$ and ≤ 40 is considered moderate and > 40 is considered severe (Beck (2006)). A shift table from baseline to week 52 and to week 104 between the three categories will be presented by treatment arm. For patients who discontinue early, their value at their last assessment will be used for the MSSI analyses.

If a patient missed Week 52 or Week 104 due to COVID-19, his MSSI assessment will be performed at the first time the patient could attend an on-site visit after Week 52 or Week 104.

10.9 Quality of Life (EQ-5D-5L)

Descriptive statistics of the qualitative assessments regarding mobility, self-care, usual activities, pain/discomfort, and anxiety/depression will be summarized at each visit by treatment group. The number and proportion of patients with no change or improvement (difference from baseline is ≥ 0) and the number and proportion of patients with a worse score (difference from baseline is < 0) will be summarized by visit.

The overall health score (from 0 to 100) will be summarized by treatment group using descriptive statistics at each visit together with the change from baseline. A statistical comparison in the change from baseline to Week 52 and to Week 104 between the two treatment groups will be made using a t-distribution for two-samples.

If a patient missed Week 52 or Week 104 due to COVID-19, their EQ-5D-5L assessment will be performed at the first time the patient could attend a site visit after Week 52 or Week 104.

10.10 Pain Medication

Prior and concomitant medications reported during the study will be coded by World Health Organization (WHO) Drug Dictionary 20160601E. Pain medication will be identified based on the classification assigned by the investigator in the eCRF. Pain medications will be summarized by the count and percentage of the patients with each medication by standardized medication name within medication class. Medication classes will be presented alphabetically; within each medication class, standardized medication names will be sorted by decreasing order of PRX-102 frequency.

The number of patients who used pain medication at any time during the study will be summarized by treatment group presenting count and percentage of patients by the number of medications they used (0, 1, 2, up to the maximum, where medications with identical standard names are counted once and 0 represents patients who did not take any pain medication during the study).

The change from baseline to the last visit (scheduled or unscheduled) in the number of different pain medications used will be examined by shift tables with the following categories for the number of pain medications used: 0, 1, and 2+. The tables will be presented by treatment arm. For patients who terminated early, the table will show their shift to their last assessment prior to discontinuation. This analysis will be based on the pain medication used on the day of the baseline visit, and the day of last visit.

Section 7.2.1 provides information on handling partial or complete missing dates of pain medications.

10.11 Fabry Clinical Events

Following Hopkin (2016), Fabry clinical events (FCE) are classified into four categories: renal, cardiac, cerebrovascular and death due to non-cardiac reasons. The adjudicated decisions will be made by the Sponsor Medical Monitor in a blinded fashion, based on reported adverse events and clinical information included in the data base. The criteria for FCE are the following:

1. Renal events;
    a. The first occurrence of either initiation or chronic dialysis (>40 days)
    b. Renal transplantation
2. Cardiac events;
    a. Cardiac related death
    b. Myocardial infarction
    c. First time congestive heart failure
    d. Atrial fibrillation
    e. Ventricular tachycardia
    f. Evidence of progressive heart disease severe enough to require a pacemaker
    g. Implantation of pacemaker
    h. Bypass surgery
    i. Coronary artery dilatation
    j. Implantation of defibrillator
3. Cerebrovascular events;
    a. Hemorrhagic or ischemic stroke
    b. Transient Ischemic Attack
4. Death non-cardiac-related

The number and percentage of patients with at least one FCE overall and for each of its components will be presented by treatment arm together with the number of events as well rate adjusted to 100 person-years defined as number of events X 100 / total exposure (in years). For the number of patients with at least one FCE, patients who had more than one type will be counted only once.

Time to 1st event will be presented graphically by Kaplan-Meier by treatment group. The time (months) will be measured from date 1st infusion and patients with no FCE will be censored at the date of last infusion. The number of days will be converted to months by multiplying by 12/365.25.

The FCE listing will show the categories by Hopkin as well as by SOC and PT and the time to the event from randomization (months).

11. ANALYSIS OF SAFETY ENDPOINTS

All analyses presented in this section will be done using the Safety set.

11.1 Exposure

Duration of exposure to study drug (months) is based on the number of days a patient received drug and is defined as

(Last day of study drug – the first day of study drug + 1)*12/365.25.

Duration of exposure (months) will be summarized using descriptive statistics by treatment arm. The analysis will be repeated for all subgroups defined in Section 7.4.

The cumulative exposure (over all patients within treatment arm) in person-months will be provided.

The number of partial or complete infusions that a patient received will be summarized by treatment arm and by location of administration (home/site). Summary of infusion duration (hours) by visit will be presented by treatment arm.

Listing of infusion should include whether the complete dose was administered or not.

11.2 Adverse Events

Adverse events (AE) will be coded by the Medical Dictionary for Drug Regulatory Activities (MedDRA) version 19.00 or higher.

Pre-treatment AEs include all AEs occurred prior to the first study drug infusion. Pre-treatment AE will only be presented in listings.

A Treatment-emergent AE (TEAE) is defined as any AE that started post–first infusion. In case that date of onset is completely unknown, the AE will be classified as TEAE. In case the date is partially known, it will be classified as TEAE unless there is evidence, from the partial info (e.g., month of AE onset is earlier than the month of first infusion), that the AE was pre-treatment.

The number and percentage of patients with at least one TEAE and the number of TEAE will be reported by treatment arm and overall for the following parameters: Any TEAE; Related (showing jointly possibly, probably or definitely related events) TEAE; Mild or moderate TEAE; Related mild or moderate TEAE; Severe TEAE; Related severe TEAE; Serious AEs; Non- Serious AEs; Related serious AE; TEAE leading to withdrawal; Related TEAE leading to withdrawal; TEAE leading to death; and Treatment related TEAE leading to death. For selected summary tables, the

number of events will be shown together with the rate per 100 person-years defined as number of events X 100 / total exposure (in years).
In the analysis of severity, patients with event classified as "Very Severe" per CTCAE severity in eCRF will be presented in the category "Severe" for analysis.
This analysis will be repeated for the following sub-groups: ADA status, gender, FD classification and region.

The overall analysis will be repeated for injection site reaction TEAEs. Injection site reactions will be identified by their SOC and PT, as defined in Table 3 and their start time that should occur within 24 hours from the infusion, following the algorithm described in Section 11.2.3.

TEAE summary tables by the MedDRA SOC and PT will present the number and percentage of patients with at least one TEAE and the number of TEAEs. These tables will be presented by treatment arm; by treatment arm and severity; and by treatment arm and relationship to study drug.

A similar table by SOC and PT will be generated for serious TEAEs by treatment group and overall and by relationship to study drug.

In summary tables by SOC and PT, SOC will be sorted alphabetically. Within SOC, PT will be sorted in a decreasing order of PRX-102 frequency.

In the summaries of severity and relationship to study drug, the most extreme value (highest severity and closest relationship to study drug) will be used for those patients who experience the same TEAE (per PT) on more than one occasion.

Missing values associated with TEAEs will be treated as missing except for causality, severity, and outcome of a TEAE, at which occurrence a "worst case" approach will be taken in the analysis. Thus:

- If causality is missing, the TEAE will be regarded as related to pegunigalsidase alfa
- If the severity is missing, the severity of the TEAE will be regarded as severe
- If the outcome is missing and the stop date is not provided, the outcome is regarded as "ongoing"

If the seriousness is missing, all efforts should be made prior to database lock to make sure that this information is available, if still missing, the worst-case scenario will be assumed.

All TEAEs will be listed (info about seriousness, severity, relationship, action taken, outcome and the TEAE day in study relative to 1st infusion).

Serious TEAE, related TEAE will be listed as well as TEAEs leading to withdrawal or death.

A listing of patient who were infected with COVID-19 will be generated. The TEAE will be identified by the following SOC, PT and LLT: SOC = Infections and infestations; PT = Corona virus infection; LLT = Corona virus infection. For the final analysis, AEs will be reviewed if need to add also reported terms for the identification.

11.2.1 Infusion Related Reactions (IRR)

IRRs are those TEAEs which occur during the infusion or shortly after the completion of the infusion and their causality is assessed as definitely, probably, or possibly related.

Injection site reactions with SOC and PT listed in Table 3 are not considered IRR and should not be considered for the IRR analysis.

Two time-frames will be considered for the IRR analysis: during the infusions or within 2 hours after its completion (IRR-2H) or within 24 hours after its completion (IRR-24H). Classification rules for assignment of TEAEs to occur within the time frame are provided below. All IRR tables will be done on IRR-2H. Selected tables will be repeated for IRR-24H, as described below.

Table 3: Injection Site Reaction SOC and PT

MedDRA SOC	**MedDRA Preferred Term**
General disorders and administration site conditions	Infusion site discomfort
	Injection site discomfort
	Infusion site pain
	Injection site pain
	Infusion site hematoma
	Injection site hematoma
	Injection site extravasation
	Infusion site extravasation
Injury poisoning and procedural complications	Contusion
	Procedural site reaction
	Procedural pain
Vascular disorders	Vein rupture
	Poor venous access

The number and percentage of patients with at least one IRR and the number of IRRs will be reported by treatment group and overall, in an overview table with the following parameters: Any IRR; Mild or moderate IRR; Severe IRR; Serious IRR; IRR leading to withdrawal; IRR leading to death. For selected summary tables, the number of IRR will be shown together with the rate per 100 infusions defined as number of IRR X 100 / Number of Infusions.

This analysis will be repeated for IRR-24H.

This analysis will be repeated for the following sub-groups: ADA status, gender, FD classification and region.

The analysis will be also by the location of administration (Home or Site), and repeated for IRR-24H.

IRR summary tables by SOC and PT will present the number and percentage of patients with at least one IRR and the number of IRRs. The tables for the IRRs will be presented overall, by severity and by seriousness. The tables by SOC and PT will be repeated for IRR-24H.

In summary tables by SOC and PT, SOC will be sorted alphabetically. Within SOC, PT will be sorted in a decreasing order of PRX-102 frequency.

IRR-2H and IRR-24H will be listed.

11.2.2 TEAE Occurring During the Infusion or Within 2 Hours After the Infusion

To determine whether a TEAE occurred within this time frame, information collected in two eCRF pages will be considered: AE form (fields of onset date and time) and Drug Administration form (fields of administration date; start and end times; question 1 "Did the patient experience an AE during or after the infusion?" and the sub-questions in case the answer is Yes. The sub-questions are 1a: "During the infusion", 1b: "Within 2 hours after the infusion" or 1c: "Up to 24 hours after the infusion"). Events which meet one of the following criteria will be considered to occur during infusion or within 2 hours after the infusion:

- Date and time for both TEAE and infusion are complete, and the onset of the TEAE is during the infusion or within 2 hours from its completion (stop time), regardless of the answer to question 1 above;
- The answer to question 1 above is Yes, and the options selected are either 1a or 1b, and the AE is linked to the drug administration form.

All other events will not be classified into this time category.

11.2.3 TEAE Occurring During the Infusion or Within 24 Hours After the Infusion

The algorithm is similar to above with a slight modification to the two conditions, as below:

- Date and time for both TEAE and infusion are complete, and the onset of the TEAE is during the infusion or up to 24 hours from its completion (stop time), regardless of the answer to question 1 above;
- The answer to question 1 above is Yes (regardless of selection of 1a, 1b or 1c) and the AE is linked to the drug administration form.

All other events will not be classified into this time category.

11.3 Vital Signs

The vital signs (systolic and diastolic blood pressure, pulse rate, body temperature, and respiratory rate) will be summarized by treatment arm at each visit. Within each visit, vital signs and change from pre-dose will be summarized for pre-dose, and nominal times of 30 minutes, 1h, 2h, 3h, 4h, 5h and 6h from start of infusion and in addition the end of observation assessment will be also presented.

In case that vital signs are taken at additional time points, or that the nominal time points are not as above, then the measurements will only be listed (e.g., if measurements are taken at 15, 30, 45, 75, 105 minutes then the measurements at 15, 45, 75 and 105 will only be listed).

11.4 Physical Examination

The number and percentage of patients with respect to physical examination results (normal / abnormal / not done) will be summarized by body system (as described in eCRF) at each visit by treatment group.

11.5 Clinical Laboratory Test Results

The following laboratory test results will be summarized by treatment arm at each visit. Continuous measures will include the change from Baseline:

1. Hematology: Hemoglobin (g/dL), Platelets (/mm3), Total white blood cell count (/uL)
2. Coagulation Profile: Partial thromboplastin time (PTT; sec), Prothrombin time (PrT; sec)
3. Vitamin D: D2 (pg/mL), D3 (pg/mL), 1,25-Dihydroxyvitamin D (pg/mL)
4. Urinalysis: Dipstick for presence of blood, Dipstick for presence of glucose, Dipstick for presence of Protein.
5. Biochemistry: Alanine transaminase (U/L), Albumin (g/dL), Alkaline phosphatase (U/L), Aspartate transaminase (U/L), Bilirubin (total) (mg/dL), Blood urea nitrogen (mg/dL), Calcium (mg/dL), Creatinine (mg/dL), Creatine phosphokinase (U/L), Cystatin c (mg/L), Gamma-glutamyl transferase (U/L), Glucose (mg/dL), Lactate dehydrogenase (U/L), Phosphate (inorganic) (mg/dL), Potassium (mEq/L), Sodium (mEq/L), Total protein (g/dL) and Uric acid (mg/dL).

Note: serum creatinine can be recorded in two units, µmol/L and mg/dL. In calculation of eGFR or summary of creatinine, the value in µmol/L needs to be converted to mg/dL using the following conversion formula: 1 mg/dL = (1/88.4) µmol/L.

Note: Serum creatinine is present in two laboratory test categories in the Study Data Tabulation Model (SDTM) (CHEMISTRY and ENZYMATE). When results from the same sample are entered at both laboratory test category, then the value is identical. In such cases, only one of them will be used in the analysis.

In case that blood sample is taken twice at the same visit, only the results of the first blood sample (based on the time the blood sample was taken) will be used in the analyses, but both will be listed.

All collected laboratory results will be listed (also parameters that are not tabulated).

Descriptive statistics for parameters for which there are test results below the level of detection and reported as '<', will not include the change from baseline analysis. For these parameters, the analysis of actual values at the visit will be based only on the values for which the true value was observed, and the summary will indicate the number of observations that were below the limit of detection. In the listings, these observations will be presented with the '<' sign.

11.6 Electrocardiography (ECG)

Descriptive statistics for selected quantitative and qualitative ECG parameters will be summarized at each visit by treatment arm. For quantitative parameters, the change from baseline will be summarized. All parameters will be listed (note that for patients diagnosed with Atrial Fibrillation, all PR values are reported as 0 in the ECG report. This value should be considered as not accessible).

1. Quantitative ECG parameters: Mean Heart Rate
2. Qualitative ECG parameters: Rhythm – Normal Sinus Rhythm (NSR), Conduction abnormalities, left ventricular hypertrophy, Supraventricular tachycardia, Premature atrial contraction, Atrial flutter, Atrial fibrillation, Premature ventricular contraction, Ventricular tachycardia, and any clinically abnormal findings (i.e., any abnormal condition as listed above). Note that ECG abnormalities and findings are reported as characteristic = Yes in the CRF,

except for Rhythm – Normal Sinus Rhythm (NSR), for which, an abnormality is reported as characteristic = No. For consistency purposes in the reporting, for this summary it has been reversed.

11.7 Anti-Drug Antibodies

A summary table for IgG and Neutralizing antibody will present the number and percentage of patients who are positive or negative by visit. In case that IgG was tested twice on the same visit (for example, due to hypersensitivity) only the 1st test will be part of the summary by visit.

The decision about ADA status at each visit is based on sequential evaluation as follows:

1. If the IgG screening is negative then ADA at that visit is reported as "negative" (and no more evaluations).
2. If the IgG screening is "Presumptive Pos", the next evaluation is the IgG Immunodepletion

a. If IgG Immunodepletion is negative then the ADA status at the visit is reported as "negative"

b. If IgG Immunodepletion is positive then the ADA status at the visit is reported as "positive"

The table will also show for IgG and Neutralizing antibody the number and percentage of patients with their overall (i.e., throughout the study) post-treatment status where post-treatment positive is defined as positive in at least one (scheduled or unscheduled) visit post baseline (Visit 1), or negative if negative at all (scheduled or unscheduled) post-baseline visits, regardless of status at baseline.

IgE is tested in case of hypersensitivity. The table will show the number and percentage of IgE positive and negative at baseline and screening (among patients who had hypersensitivity) and the IgE post-treatment status, defined as above.

Patients are considered to be treatment emergent ADA positive if they satisfy one of the following conditions:

1. Titer boosted: patients who were IgG positive to their assigned treatment arm at baseline and boosted post treatment (i.e., titer increase by at least 4-fold from baseline. See Shankar et al. 2014 and FDA Guidance for Industry, January 2019: Immunogenicity Testing of Therapeutic Protein Products — Developing and Validating Assays for Anti-Drug Antibody Detection).
2. Treatment Induced: patients who were IgG negative to their assigned treatment arm at baseline or their IgG at baseline was missing and are positive in at least one timepoint post first infusion

The number and percentage of patients who are Treatment emergent ADA (yes/no) will be presented by treatment arm. Treatment emergent status will be missing if the patient is missing all the post-baseline assessments. For treatment emergent ADA, the table will indicate to which of the two groups a patient belongs (titer boosted or treatment induced).

A shift table from ADA status at baseline (positive / negative / missing) to overall status during study (positive if positive in at least one visit post baseline, or negative if negative at all post-baseline visits or missing if missing all post baseline assessments) will be presented by treatment arm. In addition, a shift table from Baseline to Visit 53 will be presented, where only patients who have both visits will be included.

Note that only patients who tested positive for IgG ADA will be tested for ADA characterization, i.e., IgG titer, neutralizing activity and positivity to other unique PRX-102 epitopes, will be listed.

11.8 Medication

Medications reported at screening or at any point after randomization, collected in the eCRF Concomitant Medication form, will be coded by World Health Organization (WHO) Drug Dictionary 20160601E. Medications, collected at screening, whose end date was prior to first study drug intake will only be listed. All other medications will be considered concomitant medications and will be tabulated by treatment arm by medication class and standardized medication name. Medication classes will be presented alphabetically; within each medication class, standardized names will be sorted by decreasing order of PRX-102 frequency.

Listing of medications will include start and end date together with a flag to mark medications collected at screening and stopped prior to study drug treatment start.

In order to identify ACEi, ARB and pain medications taken at baseline the following rules will be considered:

- A medication whose end date was prior to first study drug intake, regardless if start date is provided or partially/completely missing, will not be considered to be taken at baseline.
- If the end date is after first study drug intake or is missing then the medication will be considered to be taken at baseline if
    - Start date is known and was prior to first study drug intake
    - If the month and/or day of start date are missing, and the year is earlier than the year of the 1st infusion
    - If the day is missing, the year is the same year as 1st infusion and the month is earlier than the month of 1st infusion.

In all other cases, it will be assumed that the drug was taken after the 1st infusion (i.e., not taken at baseline).

11.8.1 ACEi and ARB

The number of patients treated with ACEi (only), ARB (only) and both at Baseline and during the study (including Baseline) will be summarized using descriptive statistics by treatment group.

Usage of ACEi and/or ARB while on study will be identified based on the classification on the concomitant medication provided by investigator in the eCRF.

11.8.2 Infusion Premedication

Information on infusion premedication is collected on two different forms in the eCRF: in the concomitant medication eCRF form where the identification of medications used for that purpose is done by the classification provided by investigator. In addition, the drug administration form, collects information whether premedication was used or not in relation to each infusion (and if yes, the timing – before, during or before and during infusion).

Infusion premedication will be tabulated by treatment arm by medication class and standardized medication name. Medication classes will be presented alphabetically; within each medication class, standardized names will be sorted by decreasing order of PRX-102 frequency.

A summary table of the number (%) of patients with and without premedication use at each visit by treatment group will be derived based on the drug administration form.

A shift table, to identify how many patients changed the number of infusion premedication from baseline to Week 52 and Week 104 (or the last assessment before discontinuation) will be presented. The shift table will include the following categories: 0, 1, 2, 3+. In order to assess for each subject if any infusion premedication was used at baseline or at Week 52 or 104 or last assessment, the drug administration form at that visit will be used. If based on this form, no premedication was administered then the number of infusion premedication at that visit is 0. In case premedication administration at visit of interest is confirmed from drug administration form, the number of infusion premedication for each subject will be determined by the number of medications reported in the concomitant medications form in the eCRF, in which classification for infusion premedication is marked, and the date of the study drug infusion is within the start and end date of the premedication. Note that physician may change instructions for medication several days before the visit, so no window should be considered when comparing the dates. In case of missing or partially missing dates of infusion premedication, the following imputation rules should be considered before comparing the study drug infusion date to the medication start/end dates:

- When the start date is completely missing or the year is missing then it's assumed the medication was taken at baseline.
- When the end date is completely missing or in case the year is missing, it's assumed the medication was taken until the last visit (for patients who completed the study or terminated).
- For partially missing dates (but year is available): if month is missing then assume January for start date or December for end date. If day is missing then assume the 1st day of the month for the start date or the last day of the month for the end date.

Counting of premedication at each visit will be within medication class, namely, 0 means that the patient did not take any infusion premedication at the visit, a value of 2 means that the patients used infusion premeditations from 2 medication classes (regardless of the number of medications within a class).

A listing will be provided for infusion premeditations.

11.8.2.1 Infusion Premedication and IRR

The analysis in this section will be performed for IRR-2H

Each infusion will be classified based on whether infusion premedication was used for that infusion (Yes/No) and whether infusion premedication was used at baseline (Yes/No) to create 4 categories. This classifies infusions and not patients, so that different infusions for the same subjects can be classified differently for the first question above.

The following will be summarized for each category: the number of post baseline infusions and the number of patients will be presented. The number of post baseline IRR, the number (%) of post baseline infusions with at least one IRR, and the number (%) of patients with at least one IRR. This summary will be for all IRR and for severe IRR.

The analysis will be repeated for infusions in the 1st year of treatment and for infusions in the 2nd year of treatment (including extensions of treatment due to COVID-19).

11.9 Brain MRI

Brain MRI will be listed.

11.10 X-Ray

X-ray will be listed.

12. ANALYSIS OF PK ENDPOINTS

PK analysis, derivation of parameters and PK summary statistics will be described in a separate Pharmacokinetic Analysis Plan.

13. CHANGES IN PLANNED ANALYSIS

13.1 Changes from Protocol to Interim Analysis SAP

The statistical section of the protocol is short and was not meant to provide detailed information for the analysis. Some of the changes in the planned analysis of PB-102-F20 are due to knowledge and insights gained in the overall clinical program of PRX-102. The analysis of the primary endpoint and its associated sensitivity analyses were discussed and agreed by the FDA. The main changes in the analysis plan compared to protocol are as follows:

1. The following endpoints were added: Infusion Related Reaction (IRR), Fabry Clinical Events and Achievement of Fabry Kidney Disease Therapeutic Goals.
2. Analysis sets: the protocol defined that a patient who received at least one complete dose will be included in the ITT and the SAP changed this to also include at least one partial dose. The definition of the Per Protocol population was also slightly revised.
3. The primary analysis was discussed with the FDA after protocol was finalized, and the agreement is described in the SAP. In particular added clarity on the choice of mixed model used, method of estimation and covariance matrix. UPCR which is a stratification factor was added as a covariate which is in line with ICH-E9. The discussion with the FDA included sensitivity for missing data and for modeling assumptions, which is different from what was described in the protocol.
4. The protocol specified assessment of the linearity assumption via residual plots. This was changed and instead it will be assessed by the MMRM model on the change in eGFR which does not assume linearity. In case of disagreement, additional assessments will be performed post-hoc.
5. The approach for multiplicity control was changed from the protocol, and was discussed and agreed with the FDA.
6. The only shift table for laboratory value will be for ADA. No other shift tables will be performed as no other safety laboratory values are of special interest.
7. Evaluation of Acute Kidney Injury is based on AE reporting and clinical judgment of the investigator and not as described in the protocol.
8. The statistical section of the protocol stated that hypersensitivity will be analyzed as an AE of special interest. Since IRR is a wider analysis which was added (see item 1 in this list), the analysis of hypersensitivity will be part of the IRR evaluation.

13.2 Changes from Interim Analysis SAP to Final Analysis SAP

1. Following the full approval of agalsidase beta, the final analysis was changed to non-inferiority.
2. The primary analysis was changed to the two-stage with quantile regression, and the RIRS (primary analysis for the interim analysis) is now a supportive analysis. This change was discussed and agreed with the FDA. In addition to estimating the median slope, quantile regression will be used to estimate the 25th and 75th quantiles as supportive analysis.
3. In the FDA interaction, the agency suggested to remove UPCR from the model. Following that, UPCR was removed from all models (RIRS, RI, 2-stage with ANCOVA, MMRM for eGFR change from BL and for lyso-Gb3 change from BL). Quantile regression for the median which includes UPCR as a covariate is used as a sensitivity analysis.
4. All models considered as sensitivity for the primary endpoint are conducted on the PP and ITT sets.
5. In the sensitivity analyses for missing data: MI under missing not at random and tipping point which are more relevant for superiority were removed.
6. Changes were made to Section 17: code for quantile regression was added and modifications to the MI under MAR were done in light of the change in the primary model. Removal of UPCR from the models was implemented.
7. Section 16 (unblinding plan) was revised due to the unplanned discussion with the FDA regarding the primary analysis.
8. The section on simulations to support the model was removed. Simulations were provided to the FDA.
9. Text with instructions related to the interim analysis removed (e.g., handling of ongoing events at the cut-off date for the interim analysis).
10. Criteria that injection site reactions should occur within 24 hours of infusion was added to the identification of injection site reactions. The rationale for this change is that during the review of interim TFL, several events that were classified as injection site reaction based on their SOC/PT time of occurrence was remote from the time of the infusion, which was judged by the clinicians as not injection site reactions.
11. Table 3 of SOC and PT to identify injection site reactions was updated.
12. A clarification was added to the derivation of duration of treatment with fabrazyme prior to the study.
13. A derivation for LVMi was added, in case LVM is available at a visit but LVMi is missing.
14. Listing of serious TEAE and of related TEAE were added.
15. At the interim analysis, IRR were considered to occur within 2 hours. For the final analysis two-time frames of IRR are considered: within 2 hours and within 24 hours.
16. Following analysis that was done as part of the Summary of Clinical Safety (2.7.4) for the MAA, an analysis of infusion premedication and IRR-2H was added.
17. In light of the 2:1 randomization ratio, adjustment to the exposure is added when presenting number of TEAE, number of IRR and number of FCE.
18. Adding a shift table for ADA: from baseline to visit 53.
19. Since randomization often occurred about two weeks prior to 1st infusion, Kaplan-Meier curves are updated to measure time from date of 1st infusion instead of randomization. In case of no events, time should be censored at the time of last infusion.

14. LIST OF TABLES, LISTINGS AND FIGURES

The statistical tables are to be generated using SAS version 9.4 or higher. In general, the sample size (n) is to be presented as an integer; minimum, and maximum are to be presented based on the number of decimal points of the reported value and other parameters with one more decimal point. Counts will be presented as an integer and with no decimal places. The percentage will be presented to one decimal place. The p-value will be rounded to three decimal places, unless it is less than 0.001, in which case, it will be presented as <0.001.

14.1 Tables

Number	Title	Population
14.1.1	Summary of Patients Disposition	Enrolled
14.1.2	Summary of Analysis set	Randomized
14.1.3	Summary of Critical or Major Protocol Violations	ITT
14.1.4	Summary of Patients Demographic Characteristics	ITT
14.1.4.1	Summary of Patients Demographic Characteristics by Gender	ITT
14.1.4.2	Summary of Patients Demographic Characteristics by ADA Status	ITT
14.1.4.3	Summary of Patients Demographic Characteristics by FD Classification	ITT
14.1.4.4	Summary of Patients Demographic Characteristics by eGFR	ITT
14.1.4.5	Summary of Patients Demographic Characteristics by eGFR Slope	ITT
14.1.4.6	Summary of Patients Demographic Characteristics by Usage of ACEi or ARB	ITT
14.1.4.7	Summary of Patients Demographic Characteristics by Region (US Vs ex-US)	ITT
14.1.4.8	Summary of Patients Demographic Characteristics by UPCR Categories	ITT
14.1.5	Summary of Baseline Characteristics	ITT
14.1.5.1	Summary of Baseline Characteristics by Gender	ITT
14.1.5.2	Summary of Baseline Characteristics by ADA Status	ITT
14.1.5.3	Summary of Baseline Characteristics by FD Classification	ITT
14.1.5.4	Summary of Baseline Characteristics by eGFR	ITT
14.1.5.5	Summary of Baseline Characteristics by eGFR Slope	ITT
14.1.5.6	Summary of Baseline Characteristics by Usage of ACEi or ARB	ITT
14.1.5.7	Summary of Baseline Characteristics by Region	ITT
14.1.5.8	Summary of Baseline Characteristics by UPCR Categories	ITT
14.1.6	Summary of Fabry Disease Medical History	ITT
14.1.6.1	Summary of Fabry Disease Medical History by Gender	ITT
14.1.6.2	Summary of Fabry Disease Medical History by ADA Status	ITT
14.1.6.3	Summary of Fabry Disease Medical History by FD Classification	ITT
14.1.6.4	Summary of Fabry Disease Medical History by eGFR	ITT
14.1.6.5	Summary of Fabry Disease Medical History by eGFR Slope	ITT

14.1.6.6	Summary of Fabry Disease Medical History by Usage of ACEi or ARB	ITT
14.1.6.7	Summary of Fabry Disease Medical History by Region	ITT
14.1.6.8	Summary of Fabry Disease Medical History by UPCR Categories	ITT
14.1.7	Summary of Medical History	ITT
14.1.7.1	Summary of Medical History by Gender	ITT
14.1.7.2	Summary of Medical History by ADA Status	ITT
14.1.7.3	Summary of Medical History by FD Classification	ITT
14.1.7.4	Summary of Medical History by eGFR	ITT
14.1.7.5	Summary of Medical History by eGFR Slope	ITT
14.1.7.6	Summary of Medical History by Usage of ACEi or ARB	ITT
14.1.7.7	Summary of Medical History by Region	ITT
14.1.7.8	Summary of Medical History by UPCR Categories	ITT
14.1.8	Summary of Treatment Compliance	ITT
14.1.9	Compliance with Infusions and eGFR Assessments During COVID-19 Pandemic	ITT
14.2.1.1	Summary of eGFR	ITT
14.2.1.1.1	Summary of eGFR by Gender	ITT
14.2.1.1.2	Summary of eGFR by ADA Status	ITT
14.2.1.1.3	Summary of eGFR by FD Classification	ITT
14.2.1.1.4	Summary of eGFR by eGFR	ITT
14.2.1.1.5	Summary of eGFR by eGFR Slope	ITT
14.2.1.1.6	Summary of eGFR by Usage of ACEi or ARB	ITT
14.2.1.1.7	Summary of eGFR by Region	ITT
14.2.1.1.8	Summary of eGFR by UPCR Categories	ITT
14.2.1.2	Summary of eGFR	PP
14.2.1.3	Summary of eGFR excluded due to AKI	ITT
14.2.1.4.1	Primary Analysis: eGFR Slope Analysis Using Quantile Regression for the Median	ITT
14.2.1.4.2	Sensitivity Analysis: eGFR Slope Analysis Using Quantile Regression for the Median	PP
14.2.1.4.3	Sensitivity Analysis: eGFR Slope Analysis Using Quantile Regression for the Median Adjusting to UPCR	ITT
14.2.1.4.4	Sensitivity Analysis: eGFR Slope Analysis Using Quantile Regression for the Median Adjusting to UPCR	PP
14.2.1.5.1	Summary of eGFR Slopes (1st Stage of 2-Stage Sensitivity Analysis)	ITT
14.2.1.5.2	Summary of eGFR Slopes (1st Stage of 2-Stage Sensitivity Analysis)	PP
14.2.1.6.1	Supportive Analysis: eGFR Slope Analysis Using Two-Stage ANCOVA	ITT
14.2.1.6.2	Supportive Analysis: eGFR Slope Analysis Using Two-Stage ANCOVA	PP

14.2.1.7.1	Supportive Analysis: eGFR Slope Analysis Using Random Intercept Model	ITT
14.2.1.7.2	Supportive Analysis: eGFR Slope Analysis Using Random Intercept Model	PP
14.2.1.7.3	Supportive Analysis: eGFR Slope Analysis Using Random Intercept Random Slope Model	ITT
14.2.1.7.4	Supportive Analysis: eGFR Slope Analysis Using Random Intercept Random Slope Model	PP
14.2.1.8	Supportive Analysis: eGFR Change from Baseline Using MMRM	ITT
14.2.1.9	Supportive Analysis: eGFR Slope Analysis Using Quantile Regression with Multiple Imputations Under MAR	ITT
14.2.1.10.1	Supportive Analysis: eGFR Slope Analysis Using Quantile Regression for the 25th Percentile	ITT
14.2.1.10.2	Supportive Analysis: eGFR Slope Analysis Using Quantile Regression for the 25th Percentile	PP
14.2.1.10.3	Supportive Analysis: eGFR Slope Analysis Using Quantile Regression for the 75th Percentile	ITT
14.2.1.10.4	Supportive Analysis: eGFR Slope Analysis Using Quantile Regression for the 75th Percentile	PP
14.2.1.11	Sensitivity Analysis Due to Elevated Serum Creatinine: eGFR Slope Analysis Using Quantile Regression	ITT
14.2.1.12.1	eGFR Slope Analysis Using Quantile Regression for the Median by Gender	ITT
14.2.1.12.2	eGFR Slope Analysis Using Quantile Regression for the Median by ADA Status	ITT
14.2.1.12.3	eGFR Slope Analysis Using Quantile Regression for the Median by FD Classification	ITT
14.2.1.12.4	eGFR Slope Analysis Using Quantile Regression for the Median by eGFR	ITT
14.2.1.12.5	eGFR Slope Analysis Using Quantile Regression for the Median by eGFR Slope	ITT
14.2.1.12.6	eGFR Slope Analysis Using Quantile Regression for the Median by Usage of ACEi or ARB	ITT
14.2.1.12.7	eGFR Slope Analysis Using Quantile Regression for the Median by Region	ITT
14.2.1.12.8	eGFR Slope Analysis Using Quantile Regression for the Median by UPCR Categories	ITT
14.2.2.1	Kidney Function - Proportion of Patients who Achieve Therapeutic Goals	ITT
14.2.2.1.1	Kidney Function - Proportion of Patients who Achieve Therapeutic Goals by Gender	ITT
14.2.2.1.2	Kidney Function - Proportion of Patients who Achieve Therapeutic Goals by ADA Status	ITT

14.2.2.1.3	Kidney Function - Proportion of Patients who Achieve Therapeutic Goals by FD Classification	ITT
14.2.2.1.4	Kidney Function - Proportion of Patients who Achieve Therapeutic Goals by UPCR Categories	ITT
14.2.2.2	Kidney Function Therapeutic Goals - Shift Table from Baseline to Post-treatment	ITT
14.2.2.3	Kidney Function Therapeutic Goals - Shift Table from Baseline to Post-treatment	PP
14.2.3.1	Summary of Plasma Lyso-Gb3 Concentrations	ITT
14.2.3.1.1	Summary of Plasma Lyso-Gb3 Concentrations by Gender	ITT
14.2.3.1.2	Summary of Plasma Lyso-Gb3 Concentrations by ADA Status	ITT
14.2.3.2	Plasma Lyso-Gb3 Change from Baseline - MMRM	ITT
14.2.3.2.1	Plasma Lyso-Gb3 Change from Baseline – MMRM by Gender	ITT
14.2.3.2.2	Plasma Lyso-Gb3 Change from Baseline – MMRM by ADA Status	ITT
14.2.3.2.3	Plasma Lyso-Gb3 Change from Baseline – MMRM by FD Classification	ITT
14.2.3.3	Proportion of Patients whose Plasma Lyso-GB3 Improved for Different Cutoffs	ITT
14.2.3.3.1	Proportion of Patients whose Plasma Lyso-GB3 Improved for Different Cutoffs by Gender	ITT
14.2.3.3.2	Proportion of Patients whose Plasma Lyso-GB3 Improved for Different Cutoffs by ADA Status	ITT
14.2.3.3.3	Proportion of Patients whose Plasma Lyso-GB3 Improved for Different Cutoffs by FD Classification	ITT
14.2.4.1	Summary of Plasma Gb3 Concentrations	ITT
14.2.4.1.1	Summary of Plasma Gb3 Concentrations by Gender	ITT
14.2.4.1.2	Summary of Plasma Gb3 Concentrations by ADA Status	ITT
14.2.5.1	Summary of Urine Lyso-Gb3 Concentrations	ITT
14.2.5.1.1	Summary of Urine Lyso-Gb3 Concentrations by Gender	ITT
14.2.5.1.2	Summary of Urine Lyso-Gb3 Concentrations by ADA Status	ITT
14.2.6.1	Summary of Cardiac MRI by Hypertrophy Status	ITT
14.2.6.1.1	Summary of Cardiac MRI by Gender and by Hypertrophy Status	ITT
14.2.6.1.2	Summary of Cardiac MRI by FD Classification and by Hypertrophy Status	ITT
14.2.6.2	Cardiac MRI - Shift in LVMI Fibrosis Segments	ITT
14.2.6.3	Cardiac MRI – Shift in Hypertrophy Status	ITT
14.2.7	Echocardiogram Qualitative Assessments – Shift from Baseline	ITT
14.2.8.1	Summary of Short Form Brief Pain Inventory - Severity Domains	ITT
14.2.8.1.1	Summary of Short Form Brief Pain Inventory - Average Pain Over 24 Hours by Gender	ITT
14.2.8.1.2	Summary of Short Form Brief Pain Inventory - Average Pain Over 24 Hours by ADA Status	ITT

14.2.8.1.3	Summary of Short Form Brief Pain Inventory - Average Pain Over 24 Hours by FD Classification	ITT
14.2.8.2	Short Form Brief Pain Inventory - Proportion of Patients with Improvement or No Change in Average Pain Severity	ITT
14.2.8.3	Summary of Short Form Brief Pain Inventory - Interference Domains	ITT
14.2.9.1	Frequency of UPCR Categories	ITT
14.2.9.1.1	Frequency of UPCR Categories by Gender	ITT
14.2.9.1.2	Frequency of UPCR Categories by ADA Status	ITT
14.2.9.1.3	Frequency of UPCR Categories by FD Classification	ITT
14.2.9.1.4	Frequency of UPCR Categories by Usage of ACEi or ARB	ITT
14.2.9.2	UPCR Categories - Shift from Baseline to Week 52 and to Week 104	ITT
14.2.10.1.1	Summary of Stress Test Qualitative Assessments – Overall Impression	ITT
14.2.10.1.2	Summary of Stress Test Qualitative Assessments – Symptoms	ITT
14.2.10.2	Stress Test Overall Impression - Shift from Baseline	ITT
14.2.11.1	Summary of Mainz Severity Score Index (MSSI)	ITT
14.2.11.2	Mainz Severity Score Index (MSSI) Overall Score - Shift from Baseline	ITT
14.2.12.1	Summary of Quality-of-Life EQ-5D-5L Overall Health Score	ITT
14.2.12.2	Frequency of Quality-of-Life EQ-5D-5L Qualitative Assessments	ITT
14.2.12.3	Proportion of Patients with Improvement or No Change of Quality-of-Life EQ-5D-5L Qualitative Assessments	ITT
14.2.13.1	Proportion of Patients Used Pain Medications	ITT
14.2.13.2	Number of Different Pain Medications Used by Patients During the Study	ITT
14.2.13.3	Number of Patients with Pain Medication Use – Shift from Baseline to Last Visit	ITT
14.2.14	Proportion of Patients with Fabry Clinical Events	ITT
14.3.1	Summary of Exposure	Safety
14.3.1.1	Summary of Exposure by Gender	Safety
14.3.1.2	Summary of Exposure by ADA Status	Safety
14.3.1.3	Summary of Exposure by FD Classification	Safety
14.3.1.4	Summary of Exposure by eGFR	Safety
14.3.1.5	Summary of Exposure by eGFR Slope	Safety
14.3.1.6	Summary of Exposure by Usage of ACEi or ARB	Safety
14.3.1.7	Summary of Exposure by Region	Safety
14.3.1.8	Summary of Exposure by UPCR Categories	Safety
14.3.2.1	Number of Infusions Overall and by Location of Administration	Safety
14.3.2.2	Summary of Infusion Duration (Hours)	Safety
14.3.3.1	Summary of Treatment Emergent Adverse Events	Safety
14.3.3.1.1	Summary of Treatment Emergent Adverse Events by Gender	Safety

14.3.3.1.2	Summary of Treatment Emergent Adverse Events by ADA Status	Safety
14.3.3.1.3	Summary of Treatment Emergent Adverse Events by FD Classification	Safety
14.3.3.1.4	Summary of Treatment Emergent Adverse Events by Region	Safety
14.3.3.2	Summary of Injection Site Reaction	Safety
14.3.3.3	TEAE by MedDRA System Organ Class and Preferred Term	Safety
14.3.3.4	TEAE by MedDRA System Organ Class and Preferred Term and by Severity	Safety
14.3.3.5	TEAE by MedDRA System Organ Class and Preferred Term and by Relationship to Study Drug	Safety
14.3.3.6	Serious TEAE by MedDRA System Organ Class and Preferred Term	Safety
14.3.3.7	Serious TEAE by MedDRA System Organ Class and Preferred Term and by Relationship to Study Drug	Safety
14.3.3.8	Summary of IRR–2H by Location of Administration	Safety
14.3.3.8a	Summary of IRR–24H by Location of Administration	Safety
14.3.3.8.1	Summary of IRR–2H by Gender	Safety
14.3.3.8.2	Summary of IRR2H by ADA Status	Safety
14.3.3.8.3	Summary of IRR2H by FD Classification	Safety
14.3.3.8.4	Summary of IRR-2H by Region	Safety
14.3.3.9	IRR-2H by MedDRA System Organ Class and Preferred Term	Safety
14.3.3.9a	IRR-24H by MedDRA System Organ Class and Preferred Term	Safety
14.3.3.10	IRR-2H by MedDRA System Organ Class and Preferred Term and by Severity	Safety
14.3.3.10a	IRR-24H by MedDRA System Organ Class and Preferred Term and by Severity	Safety
14.3.3.11	IRR-2H by MedDRA System Organ Class and Preferred Term and by Seriousness	Safety
14.3.3.11.a	IRR-24H by MedDRA System Organ Class and Preferred Term and by Seriousness	Safety
14.3.4	Vital Signs During the Study and Change from Pre-Dose	Safety
14.3.5	Summary of Physical Examinations	Safety
14.3.6.1	Summary of Hematology Test Results	Safety
14.3.6.2	Summary of Coagulation Profile at Screening	Safety
14.3.6.3	Summary of Vitamin D at Screening	Safety
14.3.6.4	Summary of Urine Analysis Test Results	Safety
14.3.6.5	Summary of Biochemistry Test Results	Safety
14.3.7.1	Summary of Electrocardiography (ECG) Quantitative Parameters	Safety
14.3.7.2	Summary of Electrocardiography (ECG) Qualitative Parameters	Safety
14.3.8.1	Summary of Anti-Drug Antibodies	Safety
14.3.8.2	Anti-Drug Antibody Shift from Baseline	Safety
14.3.8.2a	Anti-Drug Antibody Shift from Baseline to Week 104	Safety
14.3.9.1	Summary of Concomitant Medications	Safety

14.3.9.2	Proportion of Patients who Used ACEi and/or ARB	Safety
14.3.9.3	Proportion of Patients who Used Infusion Premedication	Safety
14.3.9.4	Number of Patients with Infusion Premedication (Medication Classes) Use - Shift from Baseline	Safety
14.3.9.5	Summary of Infusion Premedication	Safety
14.3.9.6	Infusion Premedication and IRR	Safety

14.2 Figures

Number	Title	Population
15.1	Time to Discontinuation (KM)	Randomized
15.2.1.1	Mean eGFR +/-SE Over Time	ITT
15.2.1.2	eGFR Change from BL +/- SE over time	ITT
15.2.1.3	eGFR Over Time – Boxplots	ITT
15.2.1.4	eGFR Difference in Slopes – Primary and Sensitivity Analyses - Forest plot	ITT
15.2.1.5	eGFR Difference in Slopes - Subgroups - Forest Plot	ITT
15.2.2.1	Mean Plasma Lyso Gb3 +/-SE Over Time	ITT
15.2.2.2	Mean Plasma Lyso Gb3 +/-SE Over Time - Change from Baseline	ITT
15.2.2.3	Mean Plasma Lyso Gb3 +/-SE Over Time by Gender	ITT
15.2.2.4	Mean Plasma Lyso Gb3 +/-SE Over Time by Gender - Change from Baseline	ITT
15.2.3	Time to first Fabry Clinical Event (KM)	ITT

14.3 Listings

Number	Title	Population
16.2.1.1	Patient Disposition	Enrolled
16.2.1.2	Completion and Discontinuation	Randomized
16.2.1.3	Analysis Population and Reason for Exclusion	Randomized
16.2.1.4	Protocol Violations	Enrolled
16.2.1.5	Study Visits	Enrolled
16.2.1.6	Patients Demographics	Enrolled
16.2.1.7.1	Fabry Disease Medical History and Past Treatments	Enrolled
16.2.1.7.2	Fabry Disease Diagnosis and Mutations	Enrolled
16.2.1.8	Other Medical History	Enrolled
16.2.1.9	Treatment Compliance	Randomized
16.2.1.10	Compliance with Infusions and eGFR Assessments During COVID-19 Pandemic	Randomized
16.2.1.11	Dose Information (Pharmacist) (To be generated only for final analysis)	Randomized
16.2.2.1.1	eGFR	Enrolled
16.2.2.1.2	eGFR slopes and Fabry Kidney Disease Therapeutic Goals	Enrolled
16.2.2.2	Plasma Lyso-Gb3 Concentration including Change from Baseline	Enrolled
16.2.2.3	Cardiac MRI	Randomized

16.2.2.4	Echocardiogram	Randomized
16.2.2.5	Brief Pain Inventory (BPI)	Enrolled
16.2.2.6	Stress Test – Bruce Protocol	Randomized
16.2.2.7	Mainz Severity Score Index (MSSI)	Randomized
16.2.2.8	Quality of Life EQ-5D-5L	Randomized
16.2.2.9	Fabry Clinical Events	Safety
16.2.3.1	Exposure	Randomized
16.2.3.2.1	Pre-treatment Adverse Events	Randomized
16.2.3.2.2	Treatment Emergent Adverse Events	Safety
16.2.3.2.3	Treatment-Emergent Adverse Events Leading to Withdrawal or Death	Safety
16.2.3.2.4	Serious Treatment-Emergent Adverse Events	Safety
16.2.3.2.5	Related Treatment-Emergent Adverse Events	Safety
16.2.3.2.6	Infusion Related Reactions – 2H	Safety
16.2.3.2.6a	Infusion Related Reactions – 24H	Safety
16.2.3.2.7	Patients diagnosed by Covid-19	Safety
16.2.3.3	Vital Signs	Enrolled
16.2.3.4	Physical Examination	Enrolled
16.2.3.5.1	Laboratory: Anti-Drug Antibodies	Enrolled
16.2.3.5.1.1	Laboratory: Anti-Drug Antibodies: IgE	Enrolled
16.2.3.5.2	Laboratory Parameters: Hematology	Enrolled
16.2.3.5.3	Laboratory: Urine Lyso-Gb3 Concentration	Enrolled
16.2.3.5.4	Laboratory: Coagulation Profile	Enrolled
16.2.3.5.5	Laboratory: Vitamin D	Enrolled
16.2.3.5.6	Laboratory: Urinalysis (Dipstick)	Enrolled
16.2.3.5.7	Laboratory: Biochemistry, Serum Creatinine and Cystatin C	Enrolled
16.2.3.5.8	Laboratory: Spot Urine and Urinalysis (Excluding Urine Dipstick)	Enrolled
16.2.3.5.9	Laboratory: Plasma Gb3	Enrolled
16.2.3.5.10	Laboratory: Serology	Enrolled
16.2.3.5.11	Laboratories: Pregnancies	Enrolled
16.2.3.6	Electrocardiography (ECG)	Enrolled
16.2.3.7.1.1	Medications	Enrolled
16.2.3.7.1.2	Infusion Premedication	Enrolled
16.2.3.7.2	Infusion Premedication by Visit and IRR-2H	Safety
16.2.3.8	Chest X-Ray (Screening)	Enrolled
16.2.3.9	Brain MRI	Randomized
16.2.3.10	PK Concentrations	Randomized
16.2.3.11	Body Measurements	Enrolled

15. TABLES, LISTINGS AND FIGURES SHELLS

For the interim analysis this document was prepared by CROS NT.

For the final analysis this will be updated by unblinded statistician involved with discussion with FDA.

16. APPENDIX A.1 – UNBLINDED DATA ACCESS PLAN

16.1 Background

The statistical analysis plan (SAP) that was finalized on April 15th, 2021 described the planned analyses at the interim analysis (when the last patient randomized completes 12 months of treatment) and at final analysis (when the last patient randomized completes 24 months of treatment). The analyses at the interim analysis were used to support the submission of a Marketing Authorization Application (MAA) to the European Medicines Agency (EMA) with the primary analysis based on noninferiority. The SAP indicates that the final analysis will be used as a confirmatory analysis to support the re-submission for Biologics License Application (BLA) to the United States (US) Food and Drug Administration (FDA) with the primary efficacy analysis based on superiority.

Following the conversion of agalsidase beta to a full approval in March 2021, it was agreed with the FDA (End-of-Review meeting; Sep 9, 2021) that it is not necessary anymore to demonstrate superiority over agalsidase beta. Discussion with the FDA on the margin and primary analysis took place on January 21, 2022. Unblinded personnel from the Sponsor will participate in this meeting. In light of that, the Unblinded Data Access Plan is revised to reflect this change and discuss the new working model between the unblinded and blinded teams.

Since the interim analysis will be used to support a submission to EMA, a Clinical Study Report (CSR) was developed at this time, and includes treatment assignments and data listings. Since the objective of the interim analysis is to support submission, or representative of the Sponsor had to be involved, and appropriate measures were put in place to ensure confidentiality of the results and to protect the integrity of the final analysis. The CSR and other submission documents are necessarily reviewed by a selected unblinded Interim Analysis Team that will include personnel from Protalix and Chiesi and external consultants/experts. This team is responsible for creating the regulatory documents and interacting with EU regulators. This team is also involved in the interactions with the FDA, interactions that were not foreseen at the time of developing the initial statistical analysis plan. The initial plan specified that in case that such an unplanned interaction is needed, members of the unblinded Interim Analysis Team will be responsible for the content of the meeting package, participate in the meeting and have access to the minutes. To accommodate the interim analysis, it was necessary to establish a plan for maintaining the blind of all personnel directly involved in the treatment of patients in the period between the release of the interim results and the final analysis, all personnel who are involved in the assessment of the endpoints of the trial, and all personnel who will participate in the Blinded Data Review (BDR) meeting for the final data.

Firewalls were in place before the interim analysis. They are updated now due to the interactions of the unblinded team with the FDA as laid out in this document. The current document outlines the principles at a high level and each stakeholder has a more granular documentation of the processes per the role they play during the study, at the interim and final analysis. This can be either in the form of a Standard Operating Procedures (SOP) (existing or new SOPs that were constructed or was documented in other internal guidelines. As part of the confidentiality measures, all individuals with access to the unblinded data were requested to sign confidentiality forms. Each stakeholder maintains a list of the individuals who have access to unblinded data or the results, and the mechanism by which this access was granted.

Statistical programming is performed by CROS NT. The blinded statistician and statistical programmers will perform the final analysis. The unblinded statistician and statistical programmer performed the interim analysis and are part of the Interim Analysis Team.

It is important to note that regardless of the measures that were put in place, the risk for bias at the final analysis is considered low since approximately 90% of the data for the primary analyses are collected by the time of the interim analysis.

Section 16.2 describes study milestones. Section 16.3 describes the measures that are taken for the unblinded Interim Analysis Team as well as the firewalls that were put in place. Section 16.4 describes the measures that were put in place to minimize bias. Section 16.5 describes the procedures and restrictions that were put in place for the release of information at the interim analysis.

16.2 Study Milestones

Seventy-eight (78) patients were randomized into the study. The Last Patient Last Visit (LPLV) for the interim analysis was in October 2020 and for final analysis was in October 2021. The Data Base Lock (DBL) and unblinding for the interim analysis was in April 2021, and the DBL for final analysis is anticipated in the first quarter of 2022 (Table 4).

Table 4: BALANCE Interim and Final Analyses - Key Milestones

Tentative Timelines			
	Enrollment Complete	**LPLV Date**	**DBL**
Interim Analysis	September 2019	October 2020	April 2021
Final Analysis	N/A	October 2021	Q1 2022

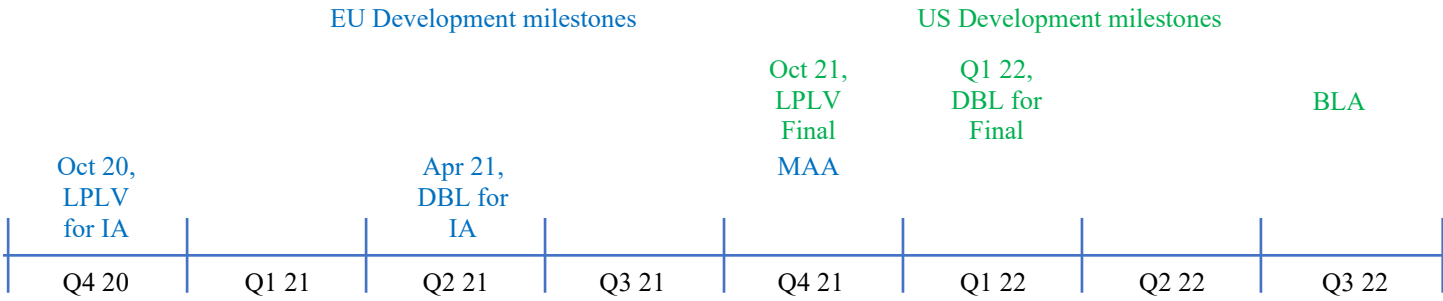
Out of the 78 patients randomized to the study, 6 (7.7%) patients have discontinued during the study. Data collection was completed for the majority of the patients (61%) at the time of the LPLV for the interim analysis. Table 5 presents the key milestones:

Table 5: Predicted Number of Patients per Milestone

	Interim Analysis	
	LPLV	**DBL and Unblinding**
Date	Oct 2020	Apr 2021
Number of patients completed 24 months of treatment or early termination	48 (61%)	60 (77)
Number of patients completed between 18- 23 months of treatment	13 (17%)	13 (17%)
Number of patients completed between 12- 17 months of treatment	17 (22%)	5 (6%)
Total	78	78

Figure 1 provides a schematic of the study with key milestones and timelines for the development programs in the EU and the US, and points out the 9-12-month period (April 2021 to Q1 2022) between the two DBL, with a 6-month period between the DBL for the interim analysis and the LPLV for the final analysis.

Figure 1: Schematic of BALANCE Development Program and Study Timelines



The primary endpoint of eGFR slope is derived from serum creatinine measurements. Serum creatinine is assessed on a monthly basis, so at the time of LPLV for the interim analysis, at least 90% of the blood samples were completed and available. At the time of DBL for the interim analysis and unblinding about 96% of blood samples were taken.

The implication of these milestones is that the risk of bias for the final analysis is low and the creation of firewalls and other measures will further reduce this risk.

16.3 Maintaining the Blind After Interim Analysis

Different stakeholders are involved in the study with different roles. Personnel involved in the study can be classified into one of the three categories:

- Blinded throughout the study (until results of final analysis are available).
- Unblinded throughout the study.
- Blinded until the interim analysis and unblinded after the interim analysis.

Figure 2 maps these individuals, within their affiliation, per the above three categories by colors. For Chiesi the figure shows the minimum list of personnel blinded until the interim analysis and unblinded after the interim analysis and the list of personnel blinded throughout the study.

In this double-blind study, all patients, investigators, site personnel, laboratories, and other personnel with direct involvement in the conduct of the study or their designees have been blinded to treatment assignment. Randomization assignments are provided automatically to unblinded site personnel in response to a randomization request by the EDC system without any human involvement or anyone other than the site pharmacist who sees the randomization codes.

Unblinded personnel throughout the study include:

- Protalix: drug supply and accountability team; Quality person
- Sites: pharmacists
- IQVIA: drug monitoring and accountability team
- Target Health: randomization code generator and validator, eCRF developers. Codes are stored in a Target Health' restricted access area.

Firewalls and SOPs, to ensure separation between personnel who are unblinded throughout the study (marked in green in the figure) and all other stakeholders (orange or red in the figure) were put into place before the beginning of the study, and are not discussed in this unblinding plan.

This unblinding plan focuses on the procedures that were put in place to protect the blinding of patients during the interval after unblinding for the interim analysis and until results from final analysis are available. The exception to this is the staff of the bioanalytical laboratory who are still blinded and no samples were analyzed for PK or immunogenicity. In order to have the ADA and PK data available at the time of the interim analysis, they were unblinded several months prior to the interim analysis.

This plan impacts personnel from Protalix, Chiesi CROS NT and consultants or other providers of expert services of Protalix and/or Chiesi (e.g., PK expert; immunogenicity consultant; statisticians etc.). The Interim Analysis Team consists of all personnel who become unblinded at the time of interim analysis and includes anyone who meets at least one of the criteria below:

- Has access to the interim CSR
- Has access to the limits of the confidence interval for the primary endpoint
- Has access to treatment assignment data
- Has access to the clinical data merged with the treatment assignment codes
- Has access to interim analysis Tables Listings Figures (TLFs)
- Has access to data from the bioanalytical lab (including ADA and PK data) or the corresponding reports
- Has access to submission documents to EMA or to briefing books submitted to the FDA

The Interim Analysis Team will consist of the following personnel:

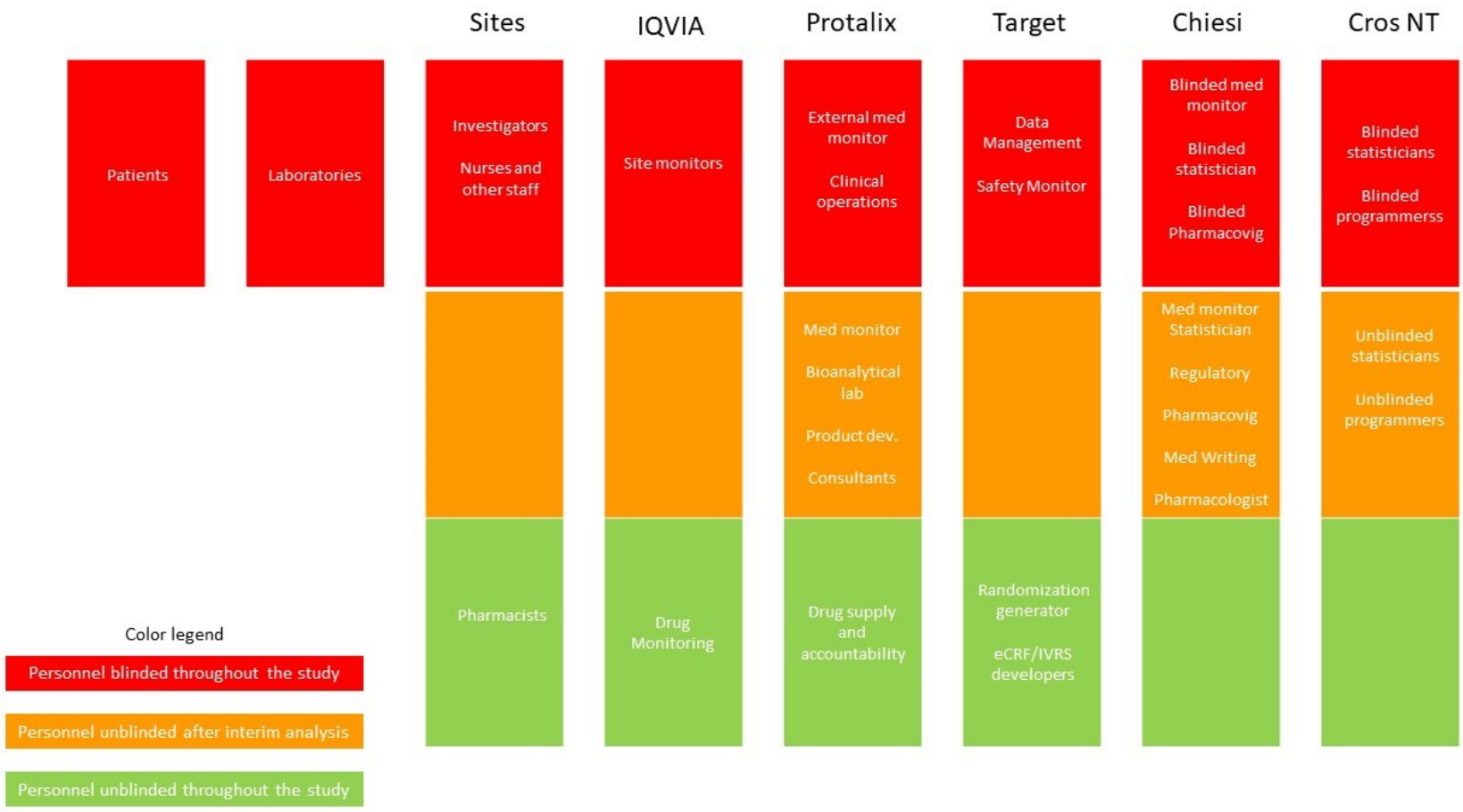
- From Protalix: medical monitor, bioanalytical laboratory personnel (including the QA and laboratory team), product development team
- From Chiesi: study team and regulatory team preparing the EMA submission (including at a minimum medical monitor, statistician, regulatory affairs, medical writer, pharmacologist and Pharmacovigilance)

- CROS NT: a team of unblinded statisticians and unblinded statistical programmers.

- Consultants to Protalix and Chiesi: this includes PK expert, immunogenicity consultant, statistician, regulatory consultants etc.

It is important to note that until the time of interim analysis, there were no restrictions or firewalls between personnel who is blinded throughout the study and personnel who was blinded until the interim analysis (with the exception of Protalix bioanalytical laboratory staff). Personnel who remain blinded after the interim analysis can support activities towards the interim DBL including data management, medical monitor, clinical operations and site monitoring.

Figure 2: Blinded and Unblinded Stakeholders



Final analysis team to support preparations towards the final DBL (including the following activities: data monitoring and cleaning, participation in blinded data review meeting, review of dry run using dummy codes, assignment of subjects to per protocol set and clinical programming) includes blinded personnel from Protalix, Target Health, CROS NT and Chiesi (Updates to SAP and shells will be done by the unblinded team as described below in Section 16.3.1):

- From Protalix: clinical operation team, medical monitoring (external consultant and an internal blinded medical monitor as backup), statistical consultant
- From Target Health: data managers
- From CROS NT: blinded statisticians and blinded statistical programmers
- From Chiesi: Blinded study team statistician (internal or Chiesi consultant); Blinded medical monitor. Other blinded members may join if there is a need.

For the medical monitoring activities, Protalix internal medical monitor was unblinded at interim analysis. In order to maintain the blind of the medical monitor for the remainder of the study, medical monitoring activities were outsourced to an external medical monitor, with a backup of a blinded internal medical monitor. There was a transition period before the interim analysis to ensure that the medical monitor is up to speed upon DBL for the interim analysis. IQVIA and the external medical monitor will continue to monitor the study conduct and be the focal point for sites and investigators. No personnel from Chiesi or CROS NT were, are or will be in touch with the investigators or sites. Protalix blinded clinical operation team is in touch with IQVIA team and if needed with the sits. No other Protalix personnel were in touch with the sites or investigators with regard to this study.

Target's blinded data management are responsible for the SDTM programming for the study, except for the ADA and PK data which are unblinding in nature. They provided SDTM data sets and dummy codes to the blinded statistician and programmer at CROS NT. The blinded statistician and programmer at CROS NT are responsible for the statistical programming for the interim and final analysis using dummy codes. The blinded statistician and statistical programmer participate in the BDR meetings before each of the two DBL. Prior to the interim analysis DBL, the blinded CROS NT team provided the SAS programs to the CROS NT unblinded statistician and statistical programmer in order to perform the interim analysis using the true randomization treatment code. Upon interim analysis DBL and approval from the Sponsor, Target Health unblinded randomization code generator uploaded the randomization codes to the restricted folder so that CROS NT unblinded statisticians and statistical programmers received the codes (This process was documented). Interim PK and ADA data were provided directly to the unblinded statistician and statistical programmers at CROS NT who were responsible for the SDTM preparation of this data as well as for the programming of all analysis involving ADA data.

Calculations of PK parameters and their analysis for interim analysis is performed by Protalix external PK consultant (who is not involved in other aspects of the study).

The responsibilities of CROS NT unblinded statisticians and statistical programmers:

- Generation of all outputs at the interim analysis
- Data from the bioanalytical laboratory (including PK and ADA data) is unblinding in nature. The bioanalytical laboratory provided the interim data directly to the unblinded team at CROS NT who was responsible for the SDTM preparations and provided the SDTM data to Protalix external PK consultant who performed the PK analysis. CROS NT unblinded team performed the ADAM and TLF programming of ADA data. The same process will be used for the final analysis.
- Perform any post-hoc and other analyses to support submission and if needed, to address Information Requests from the EMA.
- Stores unblinded datasets, randomization codes and interim analysis TLF in a restricted area, following CROS NT SOPs.
- Upon DBL for the final analysis and approval from the Sponsor, CROS NT unblinded team will provide the randomization codes to CROS NT blinded team to perform final analysis. The process will follow CROS NT internal SOPs.

After unblinding for the interim analysis, the blinded Chiesi's statistician started to manage the activities and the interactions with the blinded team in CROS NT. Protalix unblinded statistical consultant managed the activities and the interactions with the unblinded team in CROS NT.

Two BDR Meetings are planned for the studies: one prior to the interim analysis and one prior to the final DBL. The following personnel will participate in each of the meetings:

- Interim analysis
    - From CROS NT: blinded statisticians and statistical programmers
    - From Chiesi: clinical study team including statistician and medical monitor (at this point they are still blinded). The blinded statistician, medical monitor and any other blinded team member from Chiesi may participate to ensure continuation. Other blinded team members may be added if there is a need.
    - From Protalix: medical monitor (at this point still blinded) and external medical monitor (for smooth transition), blinded clinical operation personnel, blinded statistical consultant
    - From Target Health: Data management (blinded throughout the study)
    - From IQVIA: blinded CPM
- Final analysis
    - From CROS NT: blinded statisticians and programmers

- From Chiesi: blinded study team members (blinded statistician and blinded medical monitor. Other blinded team members may be added if there is a need)
- From Protalix: external medical monitor and internal blinded medical monitor (back-up medical monitor), blinded clinical operation personnel, blinded statistical consultant
- From Target: data management (blinded throughout the study)
- From IQVIA: blinded CPM

The following firewalls were put in place:

1. Within Protalix: the clinical operations team does not have access to the data, output, or treatment assignment and were not involved in the review of the CSR or other EMA submission documents or meetings with the FDA. An external medical monitor will remain blinded until the final DBL (internal backup medical monitor will remain blinded as well). The bioanalytical team became unblinded several months prior to the interim analysis in order to complete analysis of blood samples for PK and ADA on time. Protalix SOP provides instructions for the firewalls. A list of all unblinded personnel is maintained.

2. Within Target Health, a procedure to transfer codes after the DBL for the interim analysis to CROS NT with proper documentation was put in place.

3. Within Chiesi: the study and regulatory team unblinded for the interim are only involved in activities related to interim CSR, EMA submission and discussions with FDA. All the other study-related activities after unblinding for the interim analysis are managed by a blinded study team (including as a minimum a blinded statistician and a blinded medical monitor) who communicates with other blinded stakeholders managing the study (e.g., CROS NT, Protalix as needed). Interim analysis CSR, documentation for EMA submission and for discussions with FDA making reference to unblinded F20 data are stored in a restricted area with access only to authorized unblinded people. The list of all authorized unblinded personnel is maintained. A detailed separate document provides instructions and details of firewalls to ensure no dissemination of unblinding.

4. Due to COVID-19, more coordination than anticipated is required between Protalix (blinded) clinical operation and IQVIA blinded team. Only blinded team members are attending. Meeting minutes are produced to capture discussion items.

5. Data cleaning and queries after interim analysis: only blinded personnel are involved in the data cleaning.

6. Assignment of patients to the Per Protocol set is discussed during the BDR meetings. The SAP includes a list of violations that are considered major for the purpose of the Per Protocol set, in order to ensure consistency between the interim and final analyses. In the case that, during final analysis, additional violations that were not anticipated at the time of the SAP finalization will be identified, a sensitivity analysis will be performed with and without these patients in the Per Protocol analysis population.

To document firewalls and data access, each party developed an SOP / guideline outlining the approach that is used to protect the integrity of the trial and following the principles outlined above. If an appropriate SOP/guideline are already in place then they can be used. This includes Protalix, Chiesi and CROS NT.

Once the unblinding for the final analysis occurred, there are no limitations, and both the final and the interim analysis teams can work together towards the submission to the FDA.

16.3.1 Maintaining the Blind Due to Update of the SAP

Following the conversion of agalsidase beta to a full approval in March 2021, it was agreed with the FDA (End of Review Meeting; Sep 9, 2021) that it is not necessary anymore to demonstrate superiority over agalsidase beta for the final analysis, and that a discussion on the margin and primary analysis will take place in a subsequent Type C meeting with the FDA. It was agreed with the FDA that unblinded personnel will participate in this meeting. The Interim Analysis Team is involved in the meetings to support the proposed primary analysis and margin.

The following approach is taken with regard to updates to the SAP and TFL shells in order to maintain firewalls:

1. The unblinded Interim Analysis Team is responsible for preparations of material to support discussion with FDA. This also includes simulations studies and considering analytical methods discussed in recent publications.

2. The unblinded statistician is responsible to update the SAP and TFL shells. Changes between the two versions of the SAP will be documented in the final SAP. Two stages of SAP updates are planned in order to be able and advance with programming:

    - Stage 1: changes to safety and secondary efficacy endpoints following review of interim analysis TFL. No analyses performed at the interim analysis will be removed. The changes include:

        - Adding to the SAP, analyses that were done as post-hoc (e.g., IRR in 24-hour time frame).

        - Upon review of 1st draft of TFL, several changes to the SAS programs were done. These changes were documented in Note-To-File. Those changes are added to the final analysis SAP and are documented in Section 13.2.

        - Updates to footnotes of TFL.

    - Stage 2: changes to the primary analysis and sensitivity analysis, following agreement with FDA. No information on the simulations or any other unblinding information is to be included in the revised SAP.

3. The updated SAP and TFL shells will be reviewed by the unblinded Interim Analysis Team including the unblinded statistician and statistical programmer from CROS NT.

4. Updated SAP will be provided to the blinded team in order for the blinded statistician and programmer from CROS NT to start programming.

    - Note: stage 2 SAP and shells updates will be provided to the blinded team after meeting with the FDA.

5. Dry run and blinded data review meetings will be conducted by the blinded team.

16.4 Measures to Reduce Bias

The following measures were put in place in order to reduce bias after unblinding for interim analysis:

- Treatment assignments were not and will *not* be revealed to patients, investigators and other members of the site staff (e.g., nurses) until after the final DBL. Pharmacists are unblinded and are monitored by a separated unblinded monitoring team throughout the study.

- All available data at the time of the LPLV for interim analysis (including data collected beyond 12 months) was cleaned and locked before the interim analysis.

- Treatment assignments will not be revealed to the central laboratory that evaluates the serum creatinine level for eGFR calculation (it is anticipated that about 90%of the eGFR values will be included in the interim DBL).

- The use of ACEi/ARB can impact the eGFR level, so its usage is controlled before entering the study requiring all patients to be on a stable dose. Changes to ACEi/ARB usage are rare during the study. Yet, any such change will be made by the blinded external medical monitor of the study and will be reported and documented.

- All other laboratories and facilities will remain blinded with the exception of the bioanalytical laboratory for ADA and PK.

- The information that will be released to the public after the interim analysis will be limited (Section 16.5).

- The SAP will include a list of violations that are considered major for the purpose of the Per Protocol set, in order to ensure consistency before and after the interim analysis.

16.5 Release of Interim Analysis Results to the Public and Other Stakeholders

It was important to maintain the blind when the interim analysis results were released to the public and to other parties involved in the study so they remain blinded. The groups to which the results were released and the type of information that was provided to them is as follows:

- Site investigators, Key Opinion Leaders and the scientific community: were only notified if non-inferiority was demonstrated. The confidence interval, point estimate and any estimates or statistics were not provided. Basic demographic information could be provided in a pooled manner and no minimum or maximum values could be provided. Safety

information could be provided as an overview, keeping in mind that measures with few patients in either arm cannot be divulged.

- Blinded individuals within Chiesi, CROS NT and Protalix: were limited to the same level of information as the investigators and scientific communities.

- Individuals within Target and IQVIA (regardless of their blinding status prior to the interim analysis): were limited to the same level of information as the investigators and scientific communities.

- Unblinded individuals within Protalix, CROS NT and Chiesi: These individuals had full access to all information. They had to agree to maintain the blind and the firewall between the blinded and unblinded groups.

- At the time of the interim analysis, the results obtained for the analysis of the primary efficacy outcome were released to the public in a press release with the declaration of non-inferiority. The confidence interval, point estimate, and variability were not released to the public as well as the non-inferiority margin. The non-inferiority margin was in the protocol so individuals (e.g., investigators and blinded personnel within the different stakeholder had that information), yet it was not released in the press release.

- Publications (oral presentations, abstracts or manuscripts) are postponed until the final DBL.

16.6 Summary

At the time of the interim analysis to evaluate non-inferiority about 60% of BALANCE patients had reached the 24 months visit (or early terminate) and more than 90% of the eGFR values were already collected. In summary, to prevent bias for any analyses that could occur between the time of the interim cut and the last patient last visit, patients, investigators, and the site staff did not receive access to patient treatment assignments and had only knowledge of the study achieving non-inferiority. This document describes the personnel who remain blinded and the firewalls that were put in place. Persons who were unblinded at the interim analysis were required to confirm that they did not share individual treatment assignments or any other study results.

17. APPENDIX A.2 – PRIMARY, SECONDARY, SENSITIVITY AND SUPPORTIVE ANALYSES SPECIFICATIONS

17.1 Random Intercept Random Slope Specifications

The pseudo code presented in this section relates to the analysis described in Section 9.3.29.2, and may need adjustment at the time of analysis. This code to be used in the CSR will be finalized within the programming specifications.

The dependent variable, Y, is eGFR values at different time points (from the baseline visit and onwards, including unscheduled visits). The fixed part of the model includes the following terms: treatment arm (denoted by TRT with 1st level for PRX-102 and 2nd level for AGALSIDASE BETA), time (actual time of measurements in years; denoted by TIMEYR), and treatment arm by time interaction (denoted by TIMEYR*TRT).

```
proc mixed data = DATA1 method=reml alpha=0.05 covtest;
class  SUBJID TRT;
model eGFR = TRT TIMEYR TRT * TIMEYR / s ddfm = KR cl;
random INTERCEPT TIMEYR / subject=SUBJID type = UN;
estimate "PRX-102" INTERCEPT 0 TRT 0 TIMEYR 1 TRT*TIMEYR 1 0
/ cl;
estimate "ADALSIDASE BETA" INTERCEPT 0 TRT 0 TIMEYR 1 TRT*TIMEYR 0 1
/ cl;
estimate "EFFECT" INTERCEPT 0 TRT 0 TIMEYR 0 TRT*TIMEYR 1 -1
/ cl;
run;
```

SUBJID denotes the patient ID, DATA1 represent the data to be used for the analysis. Non-inferiority will be assessed based on the lower bound of the confidence interval obtained from the estimate statement labelled as “EFFECT”.

17.2 Random Intercept Model Specifications

The pseudo code presented in this section relates to the analysis described in Section 9.3.4 and may need adjustment at the time of analysis. Variables names are as defined in Section 17.1.

```
proc mixed data = DATA1 method=reml alpha=0.05 covtest empirical;
class  SUBJID TRT;
model eGFR = TRT TIMEYR TRT * TIMEYR / s cl;
random INTERCEPT / subject=SUBJID;
estimate "PRX-102" INTERCEPT 0 TRT 0 TIMEYR 1 TRT*TIMEYR 1 0
/ cl;
estimate "AGALSIDASE BETA" INTERCEPT 0 TRT 0 TIMEYR 1 TRT*TIMEYR 0  1
/ cl;
estimate "EFFECT" INTERCEPT 0 TRT 0 TIMEYR 0 TRT*TIMEYR 1 -1
/ cl;
run;
```

17.3 MMRM Model for Change from Baseline - Specifications

The efficacy analysis is based on a longitudinal mixed model for the change from Baseline in eGFR. The pseudo code presented in this section relates to the analysis described in Section 9.3.59.3.5 and may need adjustment at the time of analysis. In particular, we would confirm the ESTIMATE instructions by LSMEANS.

The dependent variable, Y, will be the change from Baseline in eGFR values at the different visits, denoted below by eGFR_CH. The fixed part of the model will include the following terms: treatment arm (denoted by TRT), visit (denoted by VISIT; class variable based on visits and will include only scheduled visits), and treatment arm by visit interaction (denoted by VISIT*TRT) and eGFR0 to represent the eGFR at Baseline. Only observations at scheduled visits or observations that were mapped to a scheduled visit (See Section 9.1) will be included in the analysis. The pseudo-code presented here was modified compared to the version submitted to FDA in Sep 2020 by adding the lsmeans statement.

```
proc mixed data = DATA1S method=reml alpha=0.05 covtest;
class  SUBJID TRT VISIT;
model eGFR_CH = TRT VISIT VISIT * TRT eGFR0 / s ddfm = KR cl;
repeated VISIT / subject=SUBJID type = UN;
lsmeans VISIT * TRT /cl;
estimate "Change from Baseline" TRT 1 -1
      VISIT*TRT 0 0 0 0 ... 1, 0, 0, ..0
                        0 0 0 0 ... -1, 0, 0, ..0 / cl;
run;
```

DATA1S denotes the data, only at scheduled visits, at the interim analysis. The interaction contrast will be set to -1 and 1 for the visit associated with 1 year of treatment (Week 52; Visit 27) and set to -1 and 1 at the visit associated with 2 years of treatment (Week 104; Visit 53).

Non-inferiority is assessed based on the lower bound of the confidence interval obtained from the estimate statement labelled “Change from baseline”.

17.4 Two-Stage Analysis – Stage 2 Specification

In the 2nd stage of the two-stage approach, a ANCOVA model will be fitted. The pseudo code presented in this section relates to the analysis described in Section 9.3.3 9.3.3 and may need adjustment at the time of analysis.

The dependent variable, Y, will be the slope for each patient which was estimated using simple regression. The covariate is treatment arm (denoted by TRT).

```
proc mixed data = SLOPES1 method=reml alpha=0.05 covtest;
class  SUBJID TRT;
model SLOPE = TRT / s ddfm = KR cl;
lsmeans TRT/diff cl;
run;
```

SLOPES1 denotes the slopes of each patient.

Non-inferiority is assessed based on the lower bound of the confidence interval obtained from the LSMEANS statement.

17.5 Two-Stage Analysis with Quantile Regression – Specification

In the 2nd stage of the two-stage approach with quantile regression, a quantiles regression model will be fitted. The pseudo code presented in this section relates to the analysis described in Section 9.2 and may need adjustment at the time of analysis.

The interior point method will be used for estimation of the quantile and resampling will be used for estimation of the SE. The dependent variable, Y, will be the slope for each patient which was estimated using simple regression. The covariate is treatment arm (denoted by TRT).

```
proc quantreg data = SLOPES1 algorithm = interior ci = resampling;
class   TRT;
model SLOPE = TRT / quantile = 0.5 seed=1092376;
estimate ”PRX-102”                          intercept 1 TRT 0 1 /cl;
estimate ”ADAKSIDASE BETA”                  intercept 1 TRT 1 0 /cl;
estimate ”PRX-102 - ADAKSIDASE BETA”        intercept 0 TRT -1 1 /cl;
run;
```

For the sensitivity analysis which includes UPCR, the stratification factor (UPCR < 1; ≥ 1 gr/gr; denoted by UPCR_STRAT) will be included in the model, as follow.

```
proc quantreg data = SLOPES1 algorithm = interior ci = resampling;
class   TRT UPCR_STRAT;
model SLOPE = TRT UPCR_STRAT / quantile = 0.5 seed=1092376;
estimate ”PRX-102”                          intercept 1 TRT 0 1 UPCR_STRAT 0.5 0.5 /cl;
estimate ”ADAKSIDASE BETA”                  intercept 1 TRT 1 0 UPCR_STRAT 0.5 0.5
/cl;
estimate ”PRX-102 - ADAKSIDASE BETA”        intercept 0 TRT -1 1 /cl;
run;
```

In order to estimate the 25th and 75th percentiles, the “quantile” in the model statement will be changed to 0.25 and 0.75.

The same seed of 1092376 will be used for all the quantile regression models.

17.6 Multiple Imputation for the Primary Analysis

MI under the MAR assumption: missing data for patients who terminated early will be imputed within each treatment arm.

MI includes 3 main steps:

1. Imputation: creating N complete datasets. We will use N = 100

2. Analysis: analyze each of the N completed data sets

3. Pooling: results of the N analyses are combined using Rubin's rule (implemented in SAS using PROC MIANALYZE), in particular a confidence interval for the effect will be provided.

The imputation part (Step 1 above) includes three sub-steps:

a) For missing eGFR, the time of planned visit will be used. No variation will be incorporated into this.

b) Create 1 dataset which satisfy monotone missingness. This will be achieved by single imputations of missing eGFR assessments that may have occurred prior to early termination. To achieve that, a simple linear regression will be fitted to the data of each patient (including scheduled and unscheduled visits) and missing eGFR at scheduled time points will be imputed using the prediction from the linear model. Time will be measured relative to day of 1st infusion.

   This approach is different than usual approach, that was also in the SAP version submitted to the FDA in Sep 2020 in which monotone missingness is achieved using Markov Chain Monte Carlo (MCMC) approach, within PROC MI with imputations done within each treatment arm. When implementing this approach prior to unblinding using dummy randomization codes, the algorithm did not converge and the reason was the large number of visits that had to be included in the model relative to the sample size. Another approach to rectify this and still using the MCMC was by imputing the missing data in several windows of time to reduce the number of visits at each such time window, however, it involved some arbitrary decisions about choice of windows. Although the approach chosen is based on single imputation, thanks to the large number of planned eGFR assessments, the low number of missing eGFR per patient and the reasonable assumption that they are MAR, it is justifiable to impute missing eGFR from subject's own data instead of borrowing data from other subjects. In case of borderline results (i.e., lower confidence bound very near the non-inferiority and/or superiority limit), additional sensitivity analysis might be performed (e.g., imputing the intermediate missing values via random draws from the distribution as predicted by the per-subject linear regression).

   For the interim analysis, most of the patients who had missing data that should be imputed to ensure monotonicity had 1 or 2 missing eGFR assessments. A small number of patients had 3 missing eGFR assessments. One subject that completed the study had 5 missing assessments, and one ongoing subject had 6 missing assessments. This approach will be

discussed, by the blinded team, prior to the final analysis and if there will be concerns about the appropriateness of this approach (e.g., too many missing assessments for a single patient), it will be re-considered.

c) Once the monotone structure is achieved, missing eGFR after early termination will be imputed using the monotone reg command. To reflect the MAR assumption, imputations will be carried out and adjusting to treatment arm. Imputations will be carried out for all patients who terminated early and imputation will be carried out up to Visit 53. The following pseudo-code will be used in this step:

```
proc MI data= OUT_IMP_DATA1 nimpute=100 round= 0.01 seed=  95234761 out =
    OUT_IMP_MAR_DATA1;
    class TRT;
    var TRT eGFR_base eGFR_V3 eGFR_V5 eGFR_V7 eGFR_V9 eGFR_V11 eGFR_V13
    eGFR_V14 eGFR_V15 eGFR_V17 eGFR_V19 eGFR_V20 eGFR_V21 eGFR_V23
    eGFR_V25 eGFR_V27 eGFR_V29 eGFR_V31 eGFR_V33 eGFR_V35 eGFR_V37
    eGFR_V39 eGFR_V40 eGFR_V41 eGFR_V43 eGFR_V45 eGFR_V47 eGFR_V49
    eGFR_V51 eGFR_V53;
    monotone reg eGFR_V3 eGFR_V5 eGFR_V7 eGFR_V9 eGFR_V11 eGFR_V13 eGFR_V14
    eGFR_V15 eGFR_V17 eGFR_V19 eGFR_V20 eGFR_V21 eGFR_V23 eGFR_V25
    eGFR_V27 eGFR_V29 eGFR_V31 eGFR_V33 eGFR_V35 eGFR_V37 eGFR_V39
    eGFR_V40 eGFR_V41 eGFR_V43 eGFR_V45 eGFR_V47 eGFR_V49 eGFR_V51
    eGFR_V53;
run;
```

In the SAP submitted to the FDA in Sep 2020, the TRT was missing from the var statement. However, in order to impute under MAR, as was planned, the code was modified.

DATA1 represents the data after achieving monotone missingness.

OUT_IMP_MAR_DATA1 will include the 100 complete datasets. Each of the completed datasets will be analyzed using the primary model (two-stage with quantile regression for the median).

```
/* Pooling of the slopes and slopes difference (contrasts from quantile regression model)  */
proc MIANALYZE parms = OUT_MAR_ESTIMATES;
    by label;
    modeleffects estimate;
    stderr stderr;
    run;
```

OUT_MAR_ESTIMATES represents the contrast estimates using the quantile regression primary model on the 100 complete data set (imputed under MAR).

17.7 MMRM Model for Change from Baseline of log Lyso Gb3

The secondary efficacy endpoint of lyso Gb3 will be analyzed using a longitudinal mixed model for the change from Baseline in log lyso Gb3. The pseudo code presented in this section relates to the analysis described in Section 10.2 and may need adjustment at the time of analysis. In

particular, we would confirm the ESTIMATE instructions by LSMEANS. For the interim analysis, available data beyond one year and up to the cutoff date will be included in the analysis.

The dependent variable, Y, will be the change from Baseline in log lyso Gb3 at the planned visits, denoted below by logLysoGB3_CH. The fixed part of the model will include the following terms: log Lyso Gb3 at Baseline (LogLysoGb3BL) treatment arm (denoted by TRT), visit (denoted by VISIT; class variable based on visits and will include only scheduled visits), and treatment arm by visit interaction (denoted by VISIT*TRT). Only observations at scheduled visits will be included in the analysis.

```
proc mixed data = DATA1S method=reml alpha=0.05 covtest;
class  SUBJID TRT VISIT;
model logLysoGb3BL = TRT VISIT VISIT * TRT  logLysoGb3BL / s ddfm = KR cl;
repeated VISIT / subject=SUBJID type = UN;
lsmeans VISIT * TRT; estimate "Log Change from Baseline" TRT 1 -1
      VISIT*TRT 0 0 0 0 ... 1, 0, 0, ..0
                        0 0 0 0 ... -1, 0, 0, ..0 / cl;
run;
```

DATA1S denotes the data, only at scheduled visits. For the final analysis, the interaction contrast will be set to -1 and 1 at the visit associated with 2 years of treatment (Week 104; Visit 53).

18. REFERENCE

Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. Archives of Internal Medicine (1916): 17 (6): 863-71.

Hao, L. and Naiman, D. (2007) Quantile Regression. Sage, London.

Hintze, J., PASS 13. NCSS, LLC. Kaysville, Utah, USA. 2014. www.ncss.com

Hopkin RJ, Cabrera G, Charrow J, et al. Risk factors for severe clinical events in male and female patients with Fabry disease treated with agalsidase beta enzyme replacement therapy: Data from the Fabry Registry. Mol Genet Metab. 2016;119(1-2):151-9

Kawel-Boehm N, et al. Normal values for cardiovascular magnetic resonance in adults and children. Journal of cardiovascular magnetic resonance. 2015: 17-29.

Koenker, R. and Bassett, G. W. (1978), “Regression Quantiles,” Econometrica, 46, 33–50.

Ortiz C, et al. Agalsidase beta treatment slows estimated glomerular filtration rate loss in classic Fabry disease patients: results from an individual patient data meta-analysis, Clinical Kidney Journal, Volume 14, Issue 4, April 2021, Pages 1136–1146.

Perry R, et al. The role of cardiac imaging in the diagnosis and management of Anderson-Fabry disease. Cardiovascular imaging. 2019: 12(7): 1230-1242

Verbeke G, Molenberghs G. Linear Mixed Models for Longitudinal Data. Wiley. 2000

Wanner C, Arad M, Baron R, et al. European Expert Consensus Statement on Therapeutic Goals in Fabry Disease. Mol Genet Metab. 2018;124(3):189-203