Statistical Analysis Plan

NCT Number: NCT04974749

Title: An Open-label Study to Evaluate the Safety, Efficacy, and Pharmacokinetics of REPLAGAL® in Treatment-naïve Chinese Subjects With Fabry Disease

Study Number: TAK-675-3001

Document Version and Date: Amendment 1.0, 01 February 2024

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STATISTICAL ANALYSIS PLAN

Study Number: *TAK-675-3001*

Study Title: *An Open-label Study to Evaluate the Safety, Efficacy, and Pharmacokinetics of REPLAGAL® in Treatment-naïve Chinese Subjects with Fabry Disease*

Phase: *3*

Version: *Amendment 1*

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Prepared by: [redacted]

Based on:

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REVISION HISTORY

Version	Approval Date	Primary Rationale for Revision
Original version	30-Sep-2022	Not Applicable
Amendment 1	1-Feb-2024	Changes due to protocol amendment and comments from dry-run

TABLE OF CONTENTS

ABBREVIATIONS

AE	adverse event
ADA	antidrug antibody
AUC	area under the curve
BP	blood pressure
BPI	brief pain inventory
bpm	beats per minute
BAb	binding antibody
BW	body weight
CKD-EPI	Chronic Kidney Disease Epidemiology
CL	serum clearance
COVID-19	coronavirus disease 2019
CPAP	Clinical Pharmacology Analysis Plan
CV	coefficient of variation
EC	ethics committee
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
EOW	every other week
IRR	infusion-related reaction
IRB	institutional review board
ITT	intention-to-treat
IV	intravenous
IVSTd	interventricular septum thickness (diastolic)
kg	kilogram
LVDd	left ventricular internal diameter (diastolic)
LVEF	left ventricular ejection fraction
LVM	left ventricular mass
LVMI	left ventricular mass index
lyso-Gb3	globotriaosylsphingosine
max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
min	minute
mL	milliliter
mmHg	millimeter mercury
MMRM	mixed effects model for repeated measures
NAb	neutralizing antibody
PD	pharmacodynamic
PK	pharmacokinetic
PP	per-protocol

PRO	patient-reported outcomes
PT	preferred term (MedDRA)
PWTd	posterior wall thickness (diastolic)
SAP	statistical analysis plan
SD	standard deviation
ss	steady state
SOC	system organ class
t	time
TEAE	treatment-emergent adverse event
U	unit
V	volume

1.0 OBJECTIVES, ENDPOINTS AND ESTIMANDS

1.1 Objectives

1.1.1 Primary Objective

The primary objective of this study is to assess the safety of REPLAGAL (0.2 mg/kg every other week (EOW) up to 52 weeks), by evaluating the incidence of serious treatment-emergent adverse events (TEAEs) over the study period, in treatment-naïve Chinese subjects with Fabry disease.

1.1.2 Secondary Objectives

The secondary objectives are as follows:

- *To evaluate other safety parameters of REPLAGAL*
- *To evaluate the efficacy of REPLAGAL on the renal parameter, estimated glomerular filtration rate (eGFR)*
- *To assess the efficacy of REPLAGAL on left ventricular mass index (LVMI) and left ventricular ejection fraction (LVEF)*
- *To evaluate the efficacy of REPLAGAL on other renal variables, pain, and pharmacodynamic (PD) markers (lyso-Gb3)*
- *To evaluate the change in hearing for subjects <18 years old*
- *To evaluate the pharmacokinetics (PK) of REPLAGAL in treatment-naïve Chinese subjects with Fabry disease*

1.1.3 Additional Objectives

Not applicable.

1.2 Endpoints

1.2.1 Primary Endpoint

Objective	***Endpoint***
To assess the safety of REPLAGAL, by evaluating the incidence of serious TEAEs over the study period, in treatment-naïve Chinese subjects with Fabry disease	• *Serious TEAEs*

1.2.2 Secondary Endpoints

1.2.2.1 Secondary Endpoints

Objective	Endpoint
To evaluate other safety parameters of REPLAGAL	• *TEAEs* • *Infusion-related reactions (IRRs)* • *Anti-drug antibody (ADA) against agalsidase alfa assessment, including neutralizing antibody (NAb) status* • *Laboratory assessments* • *Vital signs* • *Electrocardiogram (ECG) results*
To evaluate the efficacy of REPLAGAL on the renal parameter (i.e., eGFR)	• *Change from baseline at Week 52 in renal function, assessed by eGFR using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation for subjects ≥18 years old and Counahan–Barratt equation for subjects <18 years old* • *Change over time in eGFR*
To assess the efficacy of Replagal on LMVI and LVEF	• *Change over time in LVMI and LVEF as measured by echocardiography.*
To evaluate the efficacy of REPLAGAL on other renal variables, pain, and PD markers (lyso-Gb3)	• *Change over time in urine protein/creatinine ratio* • *Change over time in pain as assessed by Brief Pain Inventory (BPI) Short Form* • *Change over time in plasma lyso-Gb3 level*
To evaluate the change in hearing for subjects <18 years old	• *Change in hearing (audiology testing for subjects <18 years old at screening)*
To evaluate the PK of REPLAGAL in treatment-naïve Chinese subjects with Fabry disease	*PK parameters will include, but are not limited to, the following:* • *$AUC_{0\text{-}last}$ (min*U/mL): Area under the concentration-time curve from the time of dosing to the last measurable concentration* • *$AUC_{0\text{-}\infty}$ (min*U/mL): Area under the concentration-time curve from time zero extrapolated to infinity* • *CL (mL/min): Serum clearance of administered dose (Dose/AUC)*

- *CL (mL/min/kg): Serum clearance of administered dose normalized for body weight*
- *C_{max}: Maximum concentration observed*
- *$t_{1/2}$ (min): Terminal elimination half-life, defined as the natural log of 2 divided by the terminal rate constant (λz)*
- *t_{max} (min): Time of maximum observed concentration sampled post-dose*
- *V_{ss} (mL): Volume of distribution at steady state*
- *V_{ss} (%BW): Estimate of volume of distribution at steady state normalized for body weight*
- *AUC_{last}/dose (AUC_{last}/[U/kg]): Area under the concentration-time curve at the last sample (AUC_{last}) normalized for the dose of enzyme activity*
- *$AUC_{0-\infty}$/dose ($AUC_{0-\infty}$/[U/kg]): Area under the concentration-time curve at infinity ($AUC_{0-\infty}$) normalized for the dose of enzyme activity*
- *C_{max}/dose (C_{max}/[U/kg]): Maximum serum concentration (C_{max}) normalized for the dose of enzyme activity*

1.2.2.2 Other Secondary Endpoints

Not applicable.

1.2.3 Exploratory Endpoints

Not applicable.

1.2.4 Safety Endpoints

Safety endpoints will include the following:

- TEAEs
- Infusion-related reactions (IRRs)
- Anti-drug antibody (ADA) against agalsidase alfa assessment, including neutralizing antibody (NAb) status
- Laboratory assessments
- Vital signs
- Electrocardiogram (ECG) results

1.2.5 Other Endpoints

Not applicable

1.3 Estimand

Not applicable.

2.0 STUDY DESIGN

This is a China-only, Phase 3, multicenter, nonrandomized, open-label, single-arm study to evaluate the safety, efficacy, and PK of REPLAGAL in treatment-naïve Chinese subjects with Fabry disease. The study will consist of EOW treatment with 0.2 mg/kg IV infusion of REPLAGAL for up to 52 weeks. A total of 20 safety evaluable subjects aged ≥7 to ≤65 years are planned to be enrolled with at least 25% of the total eligible subjects in the age range of ≥7 to <18 years old.

The study will include a screening period, a baseline visit period, a treatment period, and a safety follow-up period. Screening assessments will occur from Week -7 through Week -2. Baseline visit period will be at Week -1. After completion of baseline procedures and assessments, eligible subjects will receive 0.2 mg/kg IV infusions of REPLAGAL EOW (±4 days) from Day 1 (Week 0) up to Week 52 (end of treatment [EOT] visit) during the treatment period.

Evaluation visits will be performed at clinical site at Week 8 (±4 days), Week 16 (±4 days), Week 28 (±4 days), Week 40 (±7 days), and Week 52 (±7 days). Additionally, PK assessments will occur at Day 1 (Week 0) and Week 28 (±4 days). In order to ensure subjects' safety, subjects will undergo a follow-up period of approximately 14 days (±7 days) after the completion of the treatment infusion (or the last infusion for early treatment discontinued subjects).

In the event a monitor cannot visit the site in a timely manner due to the coronavirus disease (COVID-19) pandemic, alternative monitoring approaches such as remote source data verification or telephone contact may be used to ensure data quality and integrity and maintain subject safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and permitted by the institutional review board (IRB)/ethics committee (EC), if applicable.

Safety assessments include monitoring of AEs, use of concomitant medication, clinical laboratory values, vital signs, antibody formation, physical examination, and ECG.

Efficacy assessments include measurements of eGFR, urine protein/creatine ratio, and Brief Pain Inventory (BPI). For pediatric subjects (<18 years old at screening), additional assessment for hearing will be performed.

Pharmacodynamic assessment includes plasma lyso-Gb3. Pharmacokinetic assessments will be completed by obtaining blood samples at pre-specified time points in the study. Ten subjects (5 adult subjects and 5 pediatric subjects) will provide intensive PK samples and other subjects will provide sparse PK samples.

3.0 STATISTICAL HYPOTHESES AND DECISION RULES

3.1 Statistical Hypotheses

No hypothesis will be tested.

3.2 Statistical Decision Rules

Not applicable.

3.3 Multiplicity Adjustment

Not applicable.

4.0 SAMPLE-SIZE DETERMINATION

A sample size of 20 safety evaluable subjects (safety analysis set) is planned to be enrolled in this study to descriptively provide an estimate of the serious TEAE rate. No formal sample size calculations have been done and the sample size is based on feasibility.

5.0 ANALYSIS SETS

5.1 Intent-to-treat Set

The intent-to-treat (ITT) set will include all subjects who sign the informed consent, are enrolled in the study, and have received at least 1 study drug infusion (full or partial). The ITT set will be used for demographic and disposition summaries.

5.2 Safety Analysis Set

The safety set will include all subjects in the ITT set who receive at least 1 dose of REPLAGAL. The safety set will be used for the analysis of safety endpoints.

5.3 Modified Intent-to-treat Set

The modified intent-to-treat (mITT) set will include all enrolled subjects (all subjects from the ITT) who have received at least 1 dose of investigational product and completed at least 1 post-baseline efficacy assessment of the endpoints (baseline and at least 1 efficacy post-baseline should be available). The mITT set will be used for efficacy analyses.

5.4 Per-Protocol Analysis Set

The per-protocol (PP) set will include all subjects in the mITT set excluding subjects with major protocol deviations. The PP set will be identified by a team consisting of, at a minimum, a physician and a statistician from Takeda. The PP set will be used for an efficacy sensitivity analysis.

5.5 Pharmacokinetic Analysis Set

The pharmacokinetic (PK) set will include all subjects in the ITT who receive at least 1 dose of investigational product and provide intensive or sparse PK samples. The intensive PK set will include subjects in the PK set who provide intensive sampling. Five adult subjects and 5 pediatric subjects will be included in this set. Pharmacokinetic parameters will be derived from the intensive PK set.

6.0 STATISTICAL ANALYSIS

6.1 General Considerations

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513) version 9.4 or higher.

Baseline values are defined as the last observed value before the first dose of study medication.

Means and medians will be presented to 1 more decimal place than the recorded data. The standard deviations (SDs) will be presented to 2 more decimal places than the recorded data. CIs intervals will be presented using the same number of decimal places as the parameter estimate.

Continuous variables will be summarized with descriptive statistics including the mean, standard deviation (SD), median, minimum and maximum. Categorical data will be summarized with the frequency and percentage of subjects falling within each category. Subsequently, longitudinal data will be analyzed using mixed-effects model for repeated measures (MMRM), using both the mITT and PP sets (using the PP set will serve as a sensitivity analysis).

The observed values, the change from baseline, the change over time, and the percentage change from baseline for the efficacy measurements (eGFR, urine protein/creatinine ratio, and plasma lyso-Gb3) will be summarized by sex and visit.

Subject listings will be provided for clinical outcomes from the BPI-Short Form measurement.

Audiology results will be produced at baseline and (if applicable) for each post-baseline evaluation visit.

Audiology data will also be presented in the form of individual subject listings.

6.1.1 Handling of Treatment Misallocations

Not applicable.

6.1.2 Analysis Approach for Continuous Variables

Continuous variables will be summarized with descriptive statistics including the mean, standard deviation (SD), median, minimum and maximum.

6.1.3 Analysis Approach for Binary Variables

Categorical data will be summarized with the frequency and percentage of subjects falling within each category.

6.1.4 Analysis Approach for Time-to-Event Variables

Not applicable.

6.2 Disposition of Subjects

Subject disposition for those enrolled, treated, completed, or discontinued/withdrew will be presented in summary tables using the number and percentage of subjects per category; reasons for discontinuation/withdrawal will be presented.

Subject disposition, subjects completing and prematurely discontinued during the study, and study analysis sets will be listed by subjects for all enrolled subjects.

6.3 Demographic and Other Baseline Characteristics

6.3.1 Demographics

Demographic and Baseline characteristics will be summarized using either descriptive statistics or frequency distributions, as appropriate.

The following demographic and baseline characteristics will be summarized:

- age (years) at Informed Consent
- age (years) at baseline
- age (years) at treatment start
- Fabry disease diagnosis (years) at Informed Consent
- weight (kg) and height (cm)
- BMI (kg/m^2)
- Sex
- race
- eGFR
- eGFR group at baseline (30-<60 $mL/min/1.73m^2$, 60-<90 $mL/min/1.73m^2$, 90-<135 $mL/min/1.73m^2$, ⩾135 $mL/min/1.73m^2$)
- LVMI
- LVMI group at baseline (<50 $g/m^{2.7}$, ⩾50 $g/m^{2.7}$)
- LVEF
- urine protein / creatinine ratio
- baseline pain or without pain (Yes/No)
- baseline pain severity score

- baseline pain interference score

A listing will be created to show all the demographics and baseline characteristics for each subject in the ITT and mITT. The demographic and baseline characteristic summary tables will be presented for subjects 2 to <12 years at the time of signing ICF, 12 to <18 years at the time of signing ICF, <18 years at the time of signing ICF, ≥18 years at the time of signing ICF, and the overall population.

6.3.2 Medical History and Concurrent Medical Conditions

All medical history and Fabry disease related medical history will be summarized by system organ class (SOC) and preferred term (PT). Tabulations will be presented sorted by SOC in alphabetical order and by PT within each SOC by descending frequency. Medical history will be listed by subject. Medical history will include a review of the subject's medical status and documentation of current and prior medical/surgical procedures. Medical history will include all conditions through the first dose of investigational drug.

6.3.3 Baseline Characteristics

Baseline characteristics will be summarized using either descriptive statistics or frequency distributions, as appropriate.

6.3.4 Protocol Deviations

Significant protocol deviations will be summarized in a table, as well as listed in a by-subject listing.

6.4 Medication History and Concomitant Medications

6.4.1 Prior Medications

All non-study treatment including, but not limited to, herbal treatments, vitamins, behavioral treatment, or non-pharmacological treatment, such as psychotherapy, received within 30 days prior to screening period through the first dose of investigational drug will be considered prior medication. Prior medication will be listed by subject.

The following rules will be followed in case ATC class level is missing:

- If ATC class level 4 is blank for the medication, the "Therapeutic Class (ATC Class)" will be imputed with ATC class level 3.
- If ATC class level 4 and level 3 are blank for the medication, the "Therapeutic Class (ATC Class)" will be imputed with ATC class level 2.
- If ATC class level 4, level 3 and level 2 are blank for the medication, the "Therapeutic Class (ATC Class)" will be imputed with ATC class level 1.

6.4.2 Concomitant Medications

Concomitant treatment refers to all treatment taken between the dates of the first dose of investigational product and the end of the follow-up period, inclusive. Concomitant medications will be listed by subject.

Prior and Concomitant Medication refers to all treatment taken before first dose of study treatment and still continuing after first dose of study treatment.

For missing ATC class level, the same rules will be followed as in Section 6.4.1 above.

6.5 Efficacy Analysis

6.5.1 Primary Endpoint(s) Analysis

6.5.1.1 Derivation of Endpoint

The primary analysis will be the incidence of serious TEAEs and is discussed in Section 6.6 below.

6.5.1.2 Main Analytical Approach

Not applicable.

6.5.1.3 Sensitivity Analysis

Not applicable.

6.5.1.4 Supplementary Analyses

Not applicable.

6.5.2 Secondary Endpoints Analysis

Secondary efficacy analysis

Continuous variables will be summarized with descriptive statistics including the mean, standard deviation (SD), median, minimum and maximum. Categorical data will be summarized with the frequency and percentage of subjects falling within each category. Subsequently, longitudinal data will be analyzed using mixed-effects model for repeated measures (MMRM), with covariates baseline, age and time, and an unstructured (UN) covariance structure. In case the model does not converge, this specific analysis will not be performed*. Both the mITT and PP sets (using the PP set will serve as a sensitivity analysis) will be used in these analyses.*

The observed values, the change from baseline, the change over time, and the percentage change from baseline for the efficacy measurements (eGFR, LVMI and LVEF), and urine protein/creatinine ratio will be summarized by sex and visit.

Figures of individual for the observed values of eGFR, LVMI, lyso-Gb3 and urine protein/creatinine ratio and figures of individual for the change from baseline of lyso-Gb3 and

urine protein/creatinine ratio will be generated by visit. Figures of mean (±SD) eGFR split by eGFR group at baseline and mean (±SD) LVMI split by LVMI group at baseline will be generated by visit. The observed values, the change from baseline, the change over time, and the percentage change from baseline for eGFR split by eGFR group at baseline and LVMI split by LVMI group at baseline will be summarized by sex and visit. The mITT sets will be used in these analyses.

Subject listings will be provided for clinical outcomes from the BPI-Short Form measurement. Questions from BPI short form includes 2 subscales: Pain severity and pain interference. Pain severity from questions 3 to 6 with score range 0 (no pain) to 10 (pain as bad as you can image) and pain interference from questions 9A to 9G with score 0 (does not interfere) to 10 (completely interferes). For BPI short form, impute pain severity and pain interference question scores as 0 for subjects selected Q1 as "No pain" and no corresponding answer responded for pain severity and pain interference questions. For both subscales, the average score of all questions from that subscale will be imputed if missing.

Audiology results will be produced at baseline and (if applicable) for each post-baseline evaluation visit.

Audiology data will also be presented in the form of individual subject listings.

In the analyses at week 52, only subjects who complete 52 weeks of treatment will be included.

Secondary safety analyses are discussed in Section 6.6.1.

6.5.2.1 Key Secondary Endpoint Analysis (if applicable)

Not applicable.

6.5.2.2 Derivation of Endpoints

BMI will be derived as follows:

$$\text{BMI (kg/m}^2\text{)} = \text{weight [kg]} / (\text{height [m]})^2$$

LVMI will be derived using the following formulas:

$$\text{LVM (g)} = 0.8 \times [1.04 \times \{(\text{LVDd} + \text{IVSTd} + \text{PWTd})^3 - \text{LVDd}^3\}] + 0.6,$$

where:

- LVDd is left ventricular internal diameter (diastolic) (cm),
- IVSTd is intraventricular septum thickness (diastolic) (cm),
- PWTd is posterior wall thickness (diastolic) (cm)

$$\text{LVM indexed to height (LVMI)} = \text{LVM/height}^{2.7}\ (\text{g/m}^{2.7}),$$

where height is measured in meter (m).

The height measurement at baseline will be used to derive LVMI at all timepoints for subjects ≥ 18 years. For subjects < 18 years, the height measurement closest to the LVMI assessment will be used to derive LVMI.

6.5.2.3 Main Analytical Approach

Not applicable.

6.5.2.4 Sensitivity Analysis

Longitudinal data (eGFR, urine protein/creatinine ratio, lyso-Gb3, LVMI and LVEF) will be analyzed using a mixed-effects model for repeated measures (MMRM), using the PP set.

6.5.2.5 Supplementary Analyses

Not applicable.

6.5.3 Other Secondary Endpoints Analysis (if applicable)

Not applicable.

6.5.4 Subgroup Analyses (if applicable)

Efficacy data will be split by age group (2 to <12, 12 to <18, <18, ≥18 years at Informed Consent). Lyso-Gb3 data will be split by age group (2 to <12, 12 to <18, <18, ≥18 years at Informed Consent, Total).

6.6 Safety Analysis

The primary analysis will be the incidence of serious TEAEs.

Serious adverse events will be coded using the most recent version of Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. Serious treatment-emergent adverse events (serious TEAEs) will be summarized for overall and by system organ class (SOC) and preferred term (PT). Analysis of AEs will be performed at both subject level and AE level.

Subjects will be counted once per SOC and once per PT. Multiple events of the same type will be combined for each subject and, when doing this, the worst drug relationship, severity or outcome for each event type will be presented for the analysis. When calculating event rates, the denominator will be the total population size, irrespective of dropouts over the course of follow-up.

All summaries of AE and antibody data will be split by age group (2 to <12, 12 to <18, <18, ≥18 years at Informed Consent, Total).

The safety set will be used.

6.6.1 Adverse Events

Secondary safety analysis

The secondary safety analyses will be based on the safety set.

Adverse events will be coded using the most recent version of Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary. A treatment-emergent adverse event (TEAE) is defined as any event emerging or manifesting at or after the initiation of treatment with an investigational product or medicinal product or any existing event that worsens in either intensity or frequency following exposure to the investigational product or medicinal product until the end of the safety follow-up period (14 days (+7 days) after the last infusion). Treatment-emergent adverse events (TEAEs) will be summarized for overall and by SOC and PT. Analysis of AEs will be performed at both subject level and AE level. Similar displays will be provided for IRRs. An IRR will be defined as an AE that 1) begins either during or within 24 hours after the start of the infusion and 2) is judged as related to study drug. The AEs will be summarized by severity, seriousness, and relation to investigational product.

Subjects will be counted once per SOC and once per PT. Multiple events of the same type will be combined for each subject and, when doing this, the worst severity or outcome for each event type will be presented for the analysis. When calculating event rates, the denominator will be the total population size, irrespective of dropouts over the course of follow-up.

All-cause mortality will be listed by subject. Two listings will be created – one listing the cause of death. The other listing will show serious TEAEs leading to death.

6.6.2 Adverse Events of Special Interest (if applicable)

Infusion-related reactions (IRRs) will be summarized in a similar fashion as overall TEAEs – see Section 6.6.1 above. An additional summary table will be provided for the potential relationship between IRRs and anti-agalsidase alfa antibody status. This summary table will include the specific IRR, the outcome of the IRR, and the antibody status (positive / negative).

6.6.3 Other Safety Analysis (if applicable)

Tabular summaries of other safety endpoints (eg, vital signs, blood tests, concomitant medications [coded using the World Health Organization Drug Dictionary], anti-agalsidase alfa antibody status, and infusion information) will be produced at baseline and, if applicable, for each post-baseline visit.

Changes in the results of physical examination from the baseline will be presented by visit and body system in the form of a shift table.

The observed values and the change from baseline for ECG parameters (PR, QRS, QT, QTc, and heart rate) will be summarized by visit. The number of subjects with normal and abnormal ECG results during the study period will be summarized by visit in the form of a shift table.

Laboratory data will be listed by subject. Subjects with newly occurring abnormalities outside the normal range will be flagged, listed separately, and summarized. The mean change from baseline in laboratory values or a shift table also will be provided at each visit. Change from baseline will be calculated by subtracting the baseline value from the post-baseline value.

Vital sign data will be listed by subject. Mean changes from baseline for vital sign data will be summarized. Subjects with notable abnormal values will be identified and listed separately along with their values – notable abnormal values will only be identified in adult (≥18 years) according to the following thresholds:

Sitting and supine		
Systolic BP (mmHg)	High and increase	≥140 and increase of >20 from baseline value
	Low and decrease	<90 and decrease of >20 from baseline value
Diastolic BP (mmHg)	High and increase	≥90 and increase of >15 from baseline value
	Low and decrease	<50 and decrease of >15 from baseline value
Pulse rate (bpm)	High and increase	≥100 and increase of >15 from baseline value
	Low and decrease	<45 and decrease of >15 from baseline value

The number and percentage of subjects reporting at least one use of concomitant medication during the study will be reported.

The antibody status, including binding antibody (BAb) and neutralizing antibody (NAb) status, will be summarized as categorical variable by positive and negative at baseline, by transient positive and persistent positive during the study period, and by treatment-induced positive and treatment-boosted positive at each post baseline visits. The antibody titer will be summarized as continuous variable at baseline, and by any positive, by treatment-induced positive and treatment-boosted positive each post baseline visits. *Antibody data, including* BAbs and *NAbs, will also be presented in the form of individual subject listings.*

Antibody status (including binding antibody and neutralizing antibody status) will be defined as follows:

- treatment-induced positive: pretreatment is negative, post treatment become positive
- treatment-boosted positive: pretreatment is positive, post treatment Ab titer increases ≥ 4 folds compared to pretreatment
- persistent positive: at least positive two times (>16 weeks), including last time point tested, or positive at last timepoint tested

- transient positive: at least tested positive once, last time point tested is negative

Below are more details regarding the definitions provided above:

- Treatment-induced positive: baseline negative and at least one post-baseline positive
- Treatment-boosted positive: at least one post-treatment positive with Ab titer increases ≥ 4 folds compared to pre-treatment
- Persistent and transient positive: baseline not necessarily negative
- Persistent positive: the duration between first and last post-treatment positive > 16 weeks. The last post-treatment will be the last time point tested.
- Persistent positive: This also include only if the last time point tested positive, regardless of outcome of previous tests.
- Transient positive: at least one post-treatment positive, but last time point tested is negative
- Transient positive exclude the subjects that fulfilled the condition of persistent positive.

6.6.4 Extent of Exposure and Compliance

Treatment compliance and exposure summaries will be presented. For each subject, the subject's percent of compliance, reflecting the subject's willingness to accept the intravenous infusion at each infusion visit, will be calculated as follows for up to 52 weeks:

$$\text{Compliance} = [(\text{Number of Complete Infusions Received}) / (\text{Expected Number of Infusions})] \times 100$$

Note: Expected number of infusions is defined as the number of infusions that the subject would have received up to the date of the subject's withdrawal or completion from the study. A non-complete / partial infusion is defined when the infusion has been withdrawn / stopped and not completed.

The number and percentage of subjects who received ≥80% of scheduled infusions (not including partial infusions) will be reported. In addition, the total number of missed infusions will be categorized as 0, 1, 2 to 5, and >5 and summarized by n and the percentage in each category. The number of subjects with missed infusions will also be summarized.

The duration (in months) of drug exposure to REPLAGAL will be calculated as follows:

$$\text{Exposure (months)} = [(\text{Date of the last dose} - \text{Date of first dose}) + 1]/30.4375$$

The exposure data and the total number of infusions received will be summarized by descriptive statistics. The actual dose and per protocol dose amount (mg/kg) are defined as the weight adjusted

actual dose. For actual dose amount, the dose of REPLAGAL will be based on the last available weight on or prior to each infusion date. For protocol dose amount, the first dose of REPLAGAL will be based on the subject's weight at screening. The actual and per protocol dose amounts (mg/kg) and durations of infusion (minutes) will be averaged across the non-missing infusions for each subject and then summarized by the descriptive statistics across all subjects. All exposure data will be presented in a by-subject listing.

6.7 Pharmacokinetic, Pharmacodynamic, and Biomarker Analyses

6.7.1 Pharmacokinetic Analysis

Serum PK concentrations and PK parameters will be presented according to CPAP.

6.7.2 Pharmacodynamic Analysis

The observed values, the change from baseline, the change over time, and the percentage change from baseline for the plasma lyso-Gb3 will be summarized by sex and visit.

6.7.3 Biomarker Analysis

Not applicable.

6.8 Patient Reported Outcomes (PROs) and Health Care Utilization Endpoints Analysis

6.8.1 PRO Analysis

Subject listings will be provided for clinical outcomes from the BPI-Short Form measurement.

6.8.2 Health Care Utilization Analysis

Not applicable.

6.9 Other Analyses

Not applicable.

6.10 Interim Analyses

No interim analysis will be performed.

6.11 Data Monitoring Committee/Internal Review Committee/ [Other Data Review Committees]

Not applicable.

7.0 REFERENCES

Not applicable.

8.0 CHANGES TO PROTOCOL PLANNED ANALYSES

Not applicable.

9.0 APPENDIX

9.1 Changes From the Previous Version of the SAP

SAP Section	Impacted Text (shown in bold)	Change	Rationale for Change
5.0		Update definition of ITT	Protocol amendment update
6.3.1		Add age subgroups to demographic table	Dry-run comment, add for cross reference for age subgroup analysis.
6.6.1		Update TEAE definition	Clarification
6.6.3		Add Pulse rate normal range	Clarification
6.3.1, 6.5.4, 6.6		Update age subgroups to 2-<12, 12-<18. <18, ≥18 years	Age groups are updated per EMA guidance.
6.3.1		Delete age group at baseline (<18 / ≥ 18 years) and age group at treatment start (<18 / ≥ 18 years)	Demographic table are already by age subgroups.
6.3.1		Add Fabry disease diagnosis (years) at Informed Consent, eGFR group at baseline and LVMI group at baseline	Dry-run comment, add for including Fabry disease information and detailed analysis in CSR writing.
6.3.2		Add summary for Fabry disease related medical history and over all medical history table	Dry-run comment, add for including Fabry disease information in CSR writing.
6.5.2		Add figures and supporting table	Dry-run comment, add for supporting efficacy analysis.
6.5.2		Add imputation for pain severity and pain interference question scores as 0 for subjects selected Q1 as “No pain” and no corresponding answer responded for pain severity and pain interference questions	Dry-run comment, add for supporting BPI analysis.

6.5.2		Add statement for the analyses at week 52	To avoid including early discontinued subjects in week 52 analyses.
6.5.2.2		Delete Derivation of eGFR	The study team decided to use Central lab data.
6.7.1		Update to follow CPAP	Clarification
6.3.4		Update to summary and list only significant protocol deviations.	Follow the Takeda CSR process to summary significant protocol deviations.
6.6.4		Rephrase for weight used in actual dose amount	Clarification
9.2.5		Specify the imputation rule for medical history date	Clarification
6.6.3		Add statement for BAb analysis	Dry-run comment, add for supporting antibody analysis.

9.2 Data Handling Conventions

9.2.1 General Data Reporting Conventions

Means and medians will be presented to 1 more decimal place than the recorded data. The standard deviations (SDs) will be presented to 2 more decimal places than the recorded data. CIs will be presented using the same number of decimal places as the parameter estimate. When the original data have 3 or more decimal places, the same rule as for 3 decimal places would apply to all.

For categorical variables, the count (n) and percent (%) will be displayed. Unless otherwise stated in table shells, the denominator for percentages is N (the number of subjects in the treatment group within that analysis set). For any summary by subgroups (e.g. by sex), the denominator is the number of subjects in that subgroup/treatment group within that analysis set.

- Percentages will be reported to 1 decimal place, except when the percentage equals exactly 100 where it will be displayed as an integer (100). For zero, only count and no percentage will be displayed.
- For summaries of categorical variables percentages are usually based on N as stated above. However, if missing is not a category then the denominator is the number of subjects with non-missing values. Any exceptions to the denominator should be explained in the footnotes.

9.2.2 Definition of Baseline

Baseline will be defined as the data collected prior to the first administration of study drug. If the value prior to first administration is not available, the screening value will be used.

9.2.3 Definition of Visit Windows

For the efficacy analysis, compare the unscheduled visit date to the closest infusion date greater or equal than the unscheduled visit date; if the unscheduled visit occurred on or within 4 days of that infusion date then we assign the infusion week as the visit week for the unscheduled visit. The assessment result from the unscheduled visit will be used to replace the missing result for that visit week.

9.2.4 Handling of missing data

Imputation of missing data post-baseline will be employed using last observation carried forward (LOCF) for the endpoints of the change from baseline to subsequent time points in eGFR, LVMI, LVEF, lyso-Gb3 and urine protein / creatinine ratio. No data imputation will be done for the categorical analyses of the BPI (short form).

9.2.5 Imputation of missing dates

Any date that is collected only as MONTH / YEAR, e.g, date of birth, the 15th of the month will be imputed.

If AE start date is partial, the following imputation will be done:

- If day is missing, impute 1st of the month
- If day and month are missing, impute 01 July. However, this should be checked not to be later than the AE stop date. If AE stop date is available, the 1st of the month of the AE stop month should be imputed.

If AE stop date is partial, the following imputation will be done:

- If day is missing, impute end of the month (30th or 31st)
- If day and month are missing, impute 31 Dec.

If AE stop date is missing (i.e., AE still ongoing), and AE start date has missing day and month, and imputed AE start date per above rules (01July) is after date of death (DTHDT) or end of study date (EOSDT) then “01Jan” will be imputed as AE start date.

If imputed stop date is after date of death (DTHDT) or end of study date (EOSDT), AE end date will be imputed as date of death (DTHDT) or end of study (EOSDT).

The same rules above (for AE date imputation) will be followed for prior / concomitant medications and medical history.

9.2.6 Data recorded as character values

Quantitative measurement results reported as "< X" or "<=X", i.e., below the lower limit of normal range (LLN), or "> X" or ">=X", i.e., above the upper limit of normal range (ULN), will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e., as "< X" or "> X" in the listings.

9.3 Analysis Software

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513) version 9.4 or higher.