Clinical Study Protocol

NCT Number: NCT04840667

Title: A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease

Study Number: SHP675-301

Document Version and Date: Amendment 06 (17 February 2022)

Certain information within this document has been redacted (i.e., specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

PROTOCOL: SHP675-301

TITLE:	A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease
SHORT TITLE:	A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease
STUDY PHASE:	3
ACRONYM:	Not Applicable
DRUG:	REPLAGAL® (SHP675)
IND NUMBER:	Non-IND
EUDRACT NUMBER:	2018-004689-32
OTHER NUMBER:	Not Applicable
SPONSOR:	Shire Human Genetic Therapies, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited 300 Shire Way, Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Not Applicable
PROTOCOL HISTORY:	Amendment 6: 17 February 2022 Amendment 5: 22 July 2021 Amendment 4: 24 June 2021 Amendment 3: 30 April 2021 Amendment 2: 21 January 2021 Amendment 1: 01 April 2020 Original Protocol: 17 January 2019

SUMMARY OF CHANGES FROM PREVIOUS PROTOCOL VERSION

The table below provides an overview of the changes from the previous version (dated 22 July 2021) to the current version of the protocol (Amendment 6).

The reasons for this amendment are to correct the conversion of the protein/creatinine ratio units in exclusion criterion 3, clarify the process of rescreening of subjects with prior approval of the sponsor medical monitor, clarify early termination and End of Treatment visit procedures for subjects who discontinue the study or IP treatment, specify SAE reporting procedures, and to remove references to paper assessments as a back-up to record PRO assessments.

Grammatical, typographical, or minor edits for clarity, administrative, and general formatting revisions are not identified.

Protocol Amendment		
Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number **6**	**Amendment Date** **17 Feb 2022**	**Global**
Sections Affected by Change	**Description of Each Change**	**Rationale for Change**
Protocol Signature Page, Emergency Contact Information	Updated signatory and contact information	Personnel and procedural updates.
Section 1.1 Synopsis, Section 5.2	Corrected Exclusion Criterion #3 regarding protein-creatinine ratio	To correct the conversion after changing the urine protein/creatinine ratio units from mg/mmol to mg/mg in Amendment 1.
Section 1.1 Synopsis, Section 4.4	Updated study duration	Updated year of study start.
Table 1 (footnote g), Section 7.3, Section 8.1.3.4	Clarified procedures for subjects who discontinue treatment	To specify that subjects who discontinue treatment but remain in the study should complete the EoT assessments within 10-21 days of the subject's last dose.
Table 1 (footnote a), Section 7.3, Section 8.1.3.4	Clarified rescreening procedures	To clarify circumstances under which subjects could be rescreened, and to clarify that some procedures may not need to be repeated for rescreening.
Section 6.7, Section 7.1, Section 8.1.1	Clarified that rescreening of subjects requires prior approval by sponsor medical monitor and this process is not limited solely to COVID-19 related issues	To ensure subjects appropriate for the study are not excluded.

Protocol Amendment		
Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number **6**	**Amendment Date** **17 Feb 2022**	**Global**
Sections Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 6.2.3, Section 6.7, Section 8.2.4.3, Appendix 5	Removed references to paper back-up assessments for PRO data collection if patient would not be able to attend clinic visit. Added new text about web-based data collection in case of device unavailability.	Paper back-ups were added incorrectly; they will not be used for this study
Appendix 3.4, Appendix 3.5	Changed SAE reporting process	To include and specify processes for reporting SAEs to the electronic data capture system
Appendix 3.8	Changed pregnancy reporting process	To include and specify processes for reporting pregnancy events

See Appendix 7 for protocol history, including all amendments.

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature: DocuSigned by: [redacted]	**Date:** 22-Feb-2022 | 00:10:15 JST
Clinical Science, Marketed Products Group	

Investigator's Acknowledgement

I have read this protocol for Study SHP675-301.

Title: A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please handprint or type)	

Signature: _____ **Date:** _____

EMERGENCY CONTACT INFORMATION

If a subject experiences a serious adverse event (SAE) requiring expedited reporting per the protocol, the investigator must report the event to the sponsor's Global Patient Safety Evaluation department within 24 hours of awareness, by transmitting an electronic data capture (EDC) SAE report, the preferred method of reporting SAEs.

If access to EDC is not feasible within 24 hours of receiving notice of the event, the relevant form (provided separately from this protocol):

Sponsor "Safety Report" or "Pregnancy Report" should be submitted using the fax number or email address below.

Fax number: +1-484-595-8155 (Use the email below to request a confirmation that the faxed SAE report was received.)

Email: drugsafety@shire.com

Sponsor medical monitor:

[redacted] MD, PhD, [redacted] Clinical Science

Marketed Products Group

Email: [redacted]

PRODUCT QUALITY COMPLAINTS

Investigators are required to report any investigational product quality complaints or nonmedical complaints to Shire within 24 hours. If requested, defective product(s) will be returned to the sponsor for inspection and analysis.

A product quality complaint includes any instances where there is an allegation or report relating to Shire licensed or investigational products, received in writing, electronically, or orally, which indicates an impact to a product's strength, identity, safety, purity, or quality, or which suggests that a product did not meet the criteria defined in the regulatory applications, licenses, or marketing authorizations for the product. Examples of investigational product quality complaints include, but are not limited to, the following:

Unit issues	• Capsule fill empty or overage • Bottle/vial fill shortage or overage • Capsule/tablet damaged/broken • Syringe/vial cracked/broken	• Syringe leakage • Missing components • Product discoloration • Device malfunction
Labeling	• Label missing • Leaflet or Instructions For Use (IFU) missing • Label illegible	• Incomplete, inaccurate, or misleading labeling • Lot number or serial number missing
Packaging	• Damaged packaging (eg, secondary, primary, bag/pouch) • Tampered seals • Inadequate or faulty closure	• Missing components within package
Foreign material	• Contaminated product • Particulate in bottle/vial • Particulate in packaging	

Please report the product quality complaint using the "Product Complaint Data Collection Form" via the following email address:

PQC@shire.com

For instructions on reporting AEs related to product complaints, see Appendix 3.4.

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol number: SHP675-301	**Drug:** Agalsidase alfa (REPLAGAL®; SHP675)
Title of the study: A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease	
Short title: A Phase 3, Open-label Study to Evaluate the Efficacy and Safety of REPLAGAL® in Treatment-naïve Subjects with Fabry Disease	
Study phase: 3	
Number of subjects: It is anticipated that at least 45 subjects will need to be enrolled in the study in order to obtain 36 evaluable subjects, ie, subjects who have not discontinued from investigational product and who have valid, nonmissing values of primary efficacy endpoints (estimated glomerular filtration rate [eGFR] and left ventricular mass index [LVMI]) for baseline and Week 104 assessments (completers set). In efforts to achieve equal representation, the study will target approximately 40% females and 40% males for enrollment.	
Investigator(s): This is an international, multicenter study.	
Site(s) and Region(s): This study is expected to be conducted at up to 50 sites globally.	
Study period (planned): The planned study duration is approximately 6 years from the first subject enrolled (2022).	**Clinical phase:** 3

Objectives:

Primary Objective:

The primary objective is to evaluate the efficacy of REPLAGAL (0.2 mg/kg every other week [EOW] for up to 104 weeks) on renal (eGFR) and cardiac (LVMI) parameters in subjects with a confirmed diagnosis of Fabry disease and who are naïve to any Fabry-specific treatment.

Secondary Objectives:

The secondary objectives are:

- To evaluate the efficacy of REPLAGAL 0.2 mg/kg EOW on other renal and cardiac variables and pharmacodynamic (PD) markers
- To assess the safety and tolerability of REPLAGAL over the study period.

Exploratory Objectives:

The exploratory objectives are:

- To evaluate the effect of REPLAGAL on inflammatory biomarkers relevant to Fabry disease
- To assess the effect of REPLAGAL on the subject's overall rating of pain, fatigue, severity of Fabry symptoms and quality of life
- To evaluate the effects of REPLAGAL on gastrointestinal (GI) symptoms of Fabry disease.

Rationale:

This Phase 3 study will be conducted to confirm the efficacy of REPLAGAL 0.2 mg/kg EOW for up to 104 weeks of treatment in subjects with Fabry disease who are naïve to any Fabry-specific treatment.

Investigational product, dose, and mode of administration:

REPLAGAL (agalsidase alfa) will be administered at the globally approved dose of 0.2 mg/kg EOW by intravenous (IV) infusion over 40 minutes (±10 minutes).

Methodology:

This is a global, Phase 3, multicenter, nonrandomized, open-label, single-arm, baseline-controlled study.

Eligible participants will be male and female adult subjects who are treatment naïve and who have a confirmed diagnosis of Fabry disease with left ventricular hypertrophy. Subjects with or without renal involvement may qualify for the study if their eGFR is 45 to 120 mL/min/1.73 m^2, as calculated by the Chronic Kidney Disease-Epidemiology (CKD-EPI) formula.

The study will include 1 arm, comprising subjects prospectively treated with REPLAGAL for up to 104 weeks. The change from baseline to Week 104 in eGFR and LVMI will be compared with reference values of patients with Fabry disease from the published literature (Schiffmann et al., 2009; Kampmann et al., 2008) who had similar baseline demographic and disease characteristics to the study subjects (Schiffmann et al., 2009) and who remained untreated for 2 years.

It is anticipated that at least 45 adult subjects with Fabry disease will need to be enrolled in order to obtain 36 evaluable subjects. To be considered evaluable, a subject must not have discontinued from investigational product and must have valid, nonmissing values of the primary efficacy endpoints (eGFR and LVMI) for both baseline and Week 104.

Potential subjects will participate in a screening period, which can last up to 42 days, to determine eligibility. After completion of baseline procedures and assessments, eligible subjects will receive 0.2 mg/kg REPLAGAL by IV infusion over 40 minutes EOW for 104 weeks and will have site visits approximately every 12 to 14 weeks during the 104-week treatment period. Subjects who discontinue from study treatment will continue to have study visits if they consent to do so. Approximately 14 days (-4 days/+7 days) after the last infusion or study visit, a safety follow-up contact or visit will occur to confirm the subject's safety.

Inclusion and Exclusion Criteria:

Inclusion Criteria:

The subject will not be considered eligible for the study without meeting all of the criteria below:

1. The subject must voluntarily sign an Institutional Review Board/Independent Ethics Committee/Research Ethics Board approved informed consent form after all relevant aspects of the study have been explained and discussed with the subject.
2. The subject has Fabry disease as confirmed at screening by the following criteria using a dried blood spot assay:
    - For male subjects, Fabry disease is confirmed by a deficiency of α-galactosidase A (GLA) activity and a mutation in the *GLA* gene.
    - For female subjects, Fabry disease is confirmed by a mutation in the *GLA* gene.
3. The subject is 18 to 65 years of age, inclusive.
4. Female subjects must have a negative pregnancy test at screening.
5. Female subjects of child-bearing potential must agree to use a medically acceptable method of contraception at all times during the study and for at least 14 days after the final study infusion; the methods of acceptable contraception are listed in the protocol.
6. The subject is deemed, as determined by the investigator, to have adequate general health to undergo the specified protocol-related procedures and to have no safety or medical contraindications for participation.
7. The subject has not received any treatment (approved or investigational) specific to Fabry disease, such as enzyme replacement therapy (ERT), chaperone therapy, or substrate reduction therapy.
8. The subject must have an eGFR of 45 to 120 mL/min/1.73 m^2; eGFR will be calculated by a Shire-designated laboratory using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula. If the eGFR measurement at screening is not within the stipulated range, a second eGFR measurement may be

completed and, if in range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first measurement. This inclusion criterion follows the European Guidelines for Treatment of Fabry Disease (Biegstraaten et al., 2015) and Kidney Disease Improving Global Outcomes guidelines for classification of renal disease (Kidney Disease Improving Global Outcomes [KDIGO], 2013).

9. The subject has left ventricular hypertrophy (LVH), where LVH is defined as left ventricular mass index (LVMI) >50 g/m$^{2.7}$ confirmed by cardiac magnetic resonance imaging (cMRI) at screening. The cMRI value at screening will serve as the baseline value.

Exclusion Criteria:

The subject will be excluded from the study if any of the following exclusion criteria are met:

1. In the opinion of the investigator, the subject's life expectancy is ≤5 years.
2. The subject has undergone or is scheduled to undergo kidney transplantation or is currently on dialysis, or has any signs or symptoms of end stage renal disease.
3. Urine protein/creatinine ratio (PCR) >1.5 mg/mg.
4. Subjects who have clinically relevant history of allergy or signs or symptoms of severe hypersensitivity (including hypersensitivity to the REPLAGAL active substance or any of the excipients), which in the investigator's judgment, will substantially increase the subject's risk if he or she participates in the study.
5. Cardiac fibrosis involving more than 2 segments, as determined by cMRI at screening.
6. In the opinion of the investigator, the subject has non-Fabry disease-related cause of end-organ (renal, cardiac, central nervous system) dysfunction/failure or is receiving medications that may affect the rate of disease progression, as assessed by cardiac and/or renal measures.
7. The subject has a positive test at screening for hepatitis B surface antigen, positive test for hepatitis B core antibody, positive test for hepatitis C (HCV) antibody with confirmation by HCV-ribonucleic acid polymerase chain reaction testing, or positive test for human immunodeficiency virus antibody.
8. Treatment with REPLAGAL at any time prior to the study
9. Prior treatment with any of the following medications:
   - FABRAZYME® (agalsidase beta) and its biosimilars
   - GLYSET® (miglitol)
   - ZAVESCA® (miglustat)
   - CERDELGA® (eliglustat)
   - GALAFOLD® (migalastat)
   - Any investigational product for treatment of Fabry disease
   - Chloroquine
   - Amiodarone
   - Monobenzone
   - Gentamicin.
10. The subject is pregnant or lactating.
11. The subject has a body mass index >39 kg/m^2. (BMI = kg/m^2)
12. The subject is treated or has been treated with any investigational drug within 30 days prior to study start.
13. The subject is unable to understand the nature, scope, and possible consequences of the study.
14. The subject is unable to comply with the protocol, eg, uncooperative with protocol schedule, refusal to agree to all of the study procedures, inability to return for evaluations, or is otherwise unlikely to complete the study, as determined by the investigator.

Maximum duration of subject participation in the study:

The maximum duration of participation is expected to be 112 weeks as follows:

- Planned duration of screening period: up to 42 days
- Planned duration of treatment period (baseline visit to end of treatment visit): up to 104 weeks
- Planned duration of safety follow-up period: 14 days (-4 days/+7 days).

Statistical analysis:

Endpoints and statistical analysis:

Subject Populations

- The **intent-to-treat (ITT) set** (those subjects who are considered enrolled in the study) will include all subjects who sign informed consent form and are eligible for the study based on the defined inclusion/exclusion criteria. There is a possibility that a subject is enrolled in the study but does not receive a dose of investigational product
- 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 efficacy assessment of the endpoints being analyzed.
- The **safety set** will include all subjects in the ITT set who receive at least 1 dose of investigational product. The safety set will be used for the analysis of safety endpoints.
- The **per-protocol (PP) set** will include all subjects in the ITT 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 Shire.
- The **completers set** will include all subjects in the ITT who have valid values of primary efficacy endpoints (eGFR and LVMI) for both baseline and Week 104 assessments.

Primary Efficacy Endpoints:

The primary efficacy endpoints are:

- Change from baseline at Week 104 in renal function, assessed by eGFR (using the CKD-EPI formula)
- Change from baseline at Week 104 in cardiac structure, assessed by LVMI using cMRI.

Secondary Efficacy Endpoints:

The secondary efficacy endpoints are:

- Annualized rate of change in eGFR
- Annualized rate of change in LVMI
- Change over time in eGFR
- Change over time in LVMI
- Change over time in proteinuria, measured by PCR
- Change over time of the number of fibrotic segments suggestive of cardiac fibrosis as assessed by volume of fibrosis, measured by cMRI
- Change over time in interventricular septal end-diastolic thickness and posterior wall thickness in diastole, measured by cMRI
- Change over time in plasma globotriaosylsphingosine (lyso-Gb3).

Exploratory Efficacy Endpoints:

The exploratory efficacy endpoints will include the change over time in serum levels of inflammatory biomarkers including but not limited to Interleukin 1 Beta (INTLK1β), Tumor Necrosis Factor (TNF-α), Vascular Cell Adhesion Molecule 1 (VCAM1), and Intercellular Adhesion Molecule 1 (ICAM1).

Safety Endpoints:

The safety and tolerability of REPLAGAL will be analyzed as a secondary endpoint. The safety endpoints include:

- Adverse events (AEs)
- Antidrug antibody (ADA) assessments including neutralizing antibodies (NAbs)
- Clinical laboratory tests (serum chemistry, hematology, and urinalysis)
- Electrocardiography (ECG)
- Vital signs.

Patient Reported Outcome Endpoints:

The change over time in subject rating of pain, fatigue, severity of global Fabry symptoms and quality of life will be analyzed as exploratory endpoints using the following patient-reported outcome (PRO) measures, respectively:

- The Brief Pain Inventory-Short Form
- The Brief Fatigue Inventory
- The Patient Global Impression of Fabry Symptom Severity
- The Short Form-36 Health Survey.
- The change over time in subject rating of GI symptoms as assessed by the Gastrointestinal Symptom Rating Scale.

Statistical Methodology for the Primary Efficacy Endpoints:

The primary efficacy endpoints are the change from baseline at Week 104 in eGFR and LVMI.

The primary analysis for change from baseline at Week 104 in eGFR will be based on a mixed-effects model for repeated measures (MMRM) with multiple imputation under the assumption that the missing is not at random (MNAR) using the ITT population. A Bonferroni adjustment will be used to control the overall one-sided type I error rate of 0.025 for two primary endpoints. The primary null hypothesis to be tested for eGFR is that the mean change from baseline in eGFR at Week 104 of treated subjects will be less than or equal to -6 mL/min/1.73 m^2, the reference value from untreated subjects. The alternative hypothesis is that the mean change in eGFR is greater than -6 mL/min/1.73 m^2 , the reference value of untreated subjects. A one-sided p-value to test if the mean change is greater than -6 mL/min/1.73 m^2 from MMRM model will be used to interpret that there is sufficient evidence to reject the null hypothesis. The model will include visit as the fixed effect; with adjustment for sex (male versus female), age at baseline and baseline eGFR. In the model, visit will be treated as a class variable, assuming an unstructured covariance matrix to model the within subject variability.

The primary analysis for change from baseline at Week 104 in LVMI will be based on a MMRM with imputation under the assumption that the missing is not at random using the ITT population. The primary null hypothesis to be tested for LVMI is that the mean change from baseline in LVMI at Week 104 of treated subjects will be greater than or equal to 10 $g/m^{2.7}$, the reference value from untreated subjects. The alternative hypothesis is that the mean change in LVMI is less than 10 $g/m^{2.7}$, the reference value of untreated subjects. A one-sided p-value to test if the mean change is less than 10 $g/m^{2.7}$ from MMRM model will be used to interpret that there is sufficient evidence to reject the null hypothesis. The model will include visit as the fixed effect; with adjustment for sex (male versus female), age at baseline and baseline LVMI. In the model, visit will be treated as a class variable, assuming an unstructured covariance matrix to model the within subject variability.

Missing eGFR and LVMI data due to intercurrent event or other reasons will be handled according to the treatment policy strategy.
Treatment policy strategy is defined as the strategy that considers "The occurrence of the intercurrent event is irrelevant: the value for the variable of interest is used regardless of whether or not the intercurrent event occurs." (per ICH E9 R1 Addendum). Intercurrent events can include but are not limited to the following: (1) discontinuation of study drug, (2) discontinuation of the study, or (3) initiation of a new treatment. In a situation that missingness of the eGFR or LVMI data cannot be predicted solely based on subject's observed data, such missingness will not be considered as missing at random, but rather as MNAR. The missing pattern will be evaluated and subjects with and without missing data will be compared to gain understanding of the nature of the missingness. Pattern-mixture models and Monte Carlo Markov Chain methodology will be implemented in

multiple imputation with a list of observed variables and covariates to impute missing eGFR and LVMI data with the assumption of MNAR. The covariates can be variables related to missingness identified in the comparison of subjects with and without missing data, the covariate can also be variables related to eGFR or LVMI based on medical input.

The analyses of the primary endpoints will be performed with all available data through Week 104. The repeated measure analyses will be based on the restricted maximum likelihood method assuming an unstructured covariance matrix to model the within-subject errors. If the analysis fails to converge, a compound symmetry covariance structure will be used to model the within-subject errors. Denominator degrees of freedom for the F test for fixed effects will be estimated using the Kenward-Roger approximation.

The following analyses will be performed for the primary endpoints to evaluate the robustness of the results from the primary analysis methods:

- MMRM analysis using the completers set
- MMRM analysis using the PP set.
- The eGFR and LVMI annualized rate of change and 95% CI will be estimated for subjects using the random intercept and slope model. Baseline eGFR or baseline LVMI, age at baseline, sex, and treatment duration on investigational product in year will be included in the model.
- Tipping point analysis with multiple imputation approach in ITT and mITT sets.

Descriptive summary statistics including the number of subjects (n) and mean (standard deviation [SD]) for each of raw values of the primary endpoints and change from baseline at each visit will be presented by visit.

Descriptive summary statistics for each of the primary endpoints will also be provided by sex subgroups.

Statistical Methodology for Secondary Efficacy Endpoints:

The same model used for the primary endpoints will be used.

For other secondary efficacy endpoints, summary statistics of the raw values and change from baseline will be provided descriptively by visit. Simple summary statistics will be produced initially. Continuous variables will be summarized using the following descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. The number and percentage of observed levels will be reported for all categorical measures. Subsequently, MMRM will be performed to explore the changes of the secondary efficacy endpoints over the course of follow-up. MMRM will be calculated using unstructured covariance (subject level) and restricted maximum likelihood estimation (REML). The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each secondary efficacy endpoint to account for any prespecified possible baseline confounder.

All analyses related to secondary efficacy endpoints will be performed using the ITT and mITT sets.

Statistical Methodology for Exploratory Efficacy Endpoints:

Concentrations of inflammatory biomarkers will be summarized using descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. Initially, simple summary statistics will be produced for each inflammatory biomarker. Subsequently, MMRM will be performed to explore the changes of INTLK1β, TNF-α, VCAM1, and ICAM1 over the course of follow-up.
MMRM will be built using unstructured covariance (subject level) and REML. The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each inflammatory biomarker to account for any prespecified possible baseline confounder.

All analyses related to inflammatory biomarkers will be performed using the ITT and mITT sets.

Statistical Methodology for Safety Endpoints:

All safety analyses will be presented for the safety set. Safety measures including vital signs, clinical laboratory results (hematology, chemistry, urinalysis), ECG, and ADA (including NAb) assessments will be summarized descriptively at baseline and for each postbaseline visit. Shift from baseline will be provided, if applicable**.**

Treatment emergent adverse events (TEAEs) are defined as AEs that start or deteriorate during or after the first dose of investigational product through the safety follow-up visit/contact. The number of events, incidence, and percentage of TEAEs will be summarized overall and by system organ class and preferred term at both subject level and AE level. TEAEs will be further summarized by severity and relationship to investigational product. 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 number of subjects commencing follow-up in the relevant analysis population, irrespective of dropouts during the course of follow-up. Adverse events related to investigational product including infusion-related reactions, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Statistical Methodology for Health-related Quality of Life Endpoints:

Initially, simple summary statistics will be produced for the overall score and (if available) any subscale score of each PRO measure. Continuous variables will be summarized using the following descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. The number and percentage of observed levels will be reported for all categorical measures. Subsequently, MMRM will be performed to explore the changes of PRO measures over the course of follow-up. MMRM will be calculated using unstructured covariance (subject level) and REML. The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each PRO measure to account for any prespecified possible baseline confounder.

All analyses related to PRO measures will be performed using the ITT and mITT sets.

Sample Size Justification

The primary endpoints for this study are change in eGFR and LVMI from baseline at Week 104.
Two publications by Schiffmann et al. (Schiffmann et al., 2009) and Kampmann et al. (Kampmann et al., 2008) are acknowledged as landmark references reporting on the natural history of renal and cardiac function disease progression in untreated subjects with Fabry disease, respectively. The results of these studies are used across the Fabry disease medical community as a reference for the assessment of therapeutic expectations and the response of treatment. Use of these published, aggregate data as reference values for untreated changes in the endpoints have some limitations, as the adjustment for bias in patient baseline characteristics is not feasible without access to individual patient data. Nonetheless, this relevant publicly available data will provide necessary information to evaluate the REPLAGAL efficacy benefit.

Beck et al. (Beck et al., 2015) describes the disease progression in cardiac and renal function for untreated subjects with Fabry disease based on the data reported by Schiffmann et al. (Schiffmann et al., 2009) and Kampmann et al. (Kampmann et al., 2008), respectively. In this untreated population, the mean weighted annualized rate of change for eGFR and LVMI is approximately -3 mL/min/1.73 m^2 and +5 $g/m^{2.7}$, respectively, which is equivalent to a change of -6 mL/min/1.73 m^2 and +10 $g/m^{2.7}$, respectively over a period of 104 weeks. Based on the clinical document of Summary of Clinical Efficacy provided by Shire in the REPLAGAL New Drug Submission for Health Canada, the standard deviation for change in eGFR and LVMI in ERT-naïve subjects following 24-month treatment with REPLAGAL is 7 mL/min/1.73 m^2 and 15 $g/m^{2.7}$, respectively.

The null hypothesis for eGFR is that the mean change from baseline in eGFR at Week 104 of the treated subjects is less than or equal to -6 mL/min/1.73 m^2, the mean change of untreated patients.
The alternative hypothesis states that the mean change in eGFR is greater than this reference value of -6 mL/min/1.73 m^2. The null hypothesis for LVMI is that the mean change from baseline in LVMI at Week 104 for the treated subjects is greater than or equal to +10 $g/m^{2.7}$, as the untreated patients. The alternative hypothesis states that mean change in LVMI is smaller than this reference value of +10 $g/m^{2.7}$.

The Bonferroni adjustment to control the type I error rate for the 2 primary endpoints is incorporated in the sample size determination. The Bonferroni adjustment does not make any assumptions of the primary endpoints.

For eGFR, with 36 evaluable subjects, the study has at least 90% power to detect a decrease after 104 weeks of treatment of -1.7 mL/min/1.73 m^2 compared with a reference untreated change of -6 mL/min/1.73 with SD=7 (effect size of 0.61) using a 1-sided one sample t-test at significance level of 0.0125. For LVMI, with

36 evaluable subjects, the study has at least 90% power to detect an increase after 104 weeks of treatment of 0.64 $g/m^{2.7}$ compared with a reference untreated change of 10 $g/m^{2.7}$ with SD=15 (effect size of 0.62) using a 1-sided one sample t-test at significance level of 0.0125.

It is anticipated that 45 subjects will need to be enrolled in the study in order to obtain 36 evaluable subjects, ie, subjects who have not discontinued from investigational product and who have valid, nonmissing values of primary efficacy endpoints (eGFR and LVMI) for baseline and Week 104 assessments (completers set).

1.2 Schedule of Activities

Table 1 Schedule of Activities

Study Assessments and Procedures	Screening Period[a]	Baseline Period[b]	Treatment Period[c]										Safety Follow-up Period
			EOW REPLAGAL Infusion[d] (±7 days) at (Week 2 -Week 50) and (±14 days) at (Week 52-Week 104)	Site Visits[e]									Telephone Contact/Visit[f]
	Day -50 to Day -8 (up to 42 days)	Day -7 to Day 0 (Day 0 = Week 0)[d]		Week 2 (±7 days)	Week 4 (±7 days)	Week 12 (±7 days)	Week 26 (±7 days)	Week 40 (±7 days)	Week 52 (±14 days)	Week 64 (±14 days)	Week 78 (±14 days)	Week 92 (±14 days)	Week 104 (±14 days) EOT or Early Termination[g]	14 Days (-4 days/ +7 days) from EOT or Last Infusion for Discontinued Subjects
Informed consent[h]	•													
Review of inclusion/exclusion criteria	•	•												
Confirmation of Fabry disease[i]	•													
Demography	•													
Medical history	•													
Physical examination	•						•		•		•		•	
Vital signs[j]	•	•	•	•	•	•	•	•	•	•	•	•	•	
Height[k]	•								•				•	
Weight	•	•				•	•	•	•	•	•	•	•	
Dose calculation[l]		•				•	•	•	•	•	•	•		
REPLAGAL infusion[m]		•	•	•	•	•	•	•	•	•	•	•	•[g]	
cMRI	•								•				•	
12-lead ECG	•	•					•		•		•		•	
eGFR calculation[n]	•	•				•	•	•	•	•	•	•	•	
Pregnancy test[o]	•	•	•	•	•	•	•	•	•	•	•	•	•	
Serum chemistry[p]	•	•				•	•	•	•	•	•	•	•	

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Study Assessments and Procedures	Screening Period[a]	Baseline Period[b]	Treatment Period[c]										Safety Follow-up Period
			EOW REPLAGAL Infusion[d] (±7 days) at (Week 2 -Week 50) and (±14 days) at (Week 52-Week 104)	Site Visits[e]									Telephone Contact/Visit[f]
	Day -50 to Day -8 (up to 42 days)	Day -7 to Day 0 (Day 0 = Week 0)[d]		Week 2 (±7 days)	Week 4 (±7 days)	Week 12 (±7 days)	Week 26 (±7 days)	Week 40 (±7 days)	Week 52 (±14 days)	Week 64 (±14 days)	Week 78 (±14 days)	Week 92 (±14 days)	Week 104 (±14 days) EOT or Early Termination[g]	14 Days (-4 days/ +7 days) from EOT or Last Infusion for Discontinued Subjects
Hematology[q]	•	•				•	•	•	•	•	•	•	•	
Serum inflammatory biomarkers[r]		•				•	•	•	•		•		•	
Serum ADA & NAbs[s]		•				•	•	•	•	•	•	•	•	
Plasma lyso-Gb3		•				•	•	•	•		•		•	
Urinalysis	•	•				•	•	•	•	•	•	•	•	
Proteinuria[t]	•	•				•	•	•	•	•	•	•	•	
Viral testing[u]	•													
Short Form-36 Health Survey (SF-36v2)		•				•	•	•	•	•	•	•	•	
Brief Pain Inventory Short Form (BPI-SF)		•				•	•	•	•	•	•	•	•	
Patient Global Impression of Fabry Symptom Severity (PGI-S)		•				•	•	•	•	•	•	•	•	
Brief Fatigue Inventory (BFI)		•				•	•	•	•	•	•	•	•	
Gastrointestinal Symptom Rating Scale (GSRS)		•				•	•	•	•	•	•	•	•	
Review of AEs	•	•	•	•	•	•	•	•	•	•	•	•	•	•

Table 1 Schedule of Activities

Study Assessments and Procedures	Screening Period[a]	Baseline Period[b]	Treatment Period[c]										Safety Follow-up Period
			EOW REPLAGAL Infusion[d] (±7 days) at (Week 2 -Week 50) and (±14 days) at (Week 52-Week 104)	Site Visits[e]									Telephone Contact/Visit[f]
	Day -50 to Day -8 (up to 42 days)	Day -7 to Day 0 (Day 0 = Week 0)[d]		Week 2 (±7 days)	Week 4 (±7 days)	Week 12 (±7 days)	Week 26 (±7 days)	Week 40 (±7 days)	Week 52 (±14 days)	Week 64 (±14 days)	Week 78 (±14 days)	Week 92 (±14 days)	Week 104 (±14 days) EOT or Early Termination[g]	14 Days (-4 days/ +7 days) from EOT or Last Infusion for Discontinued Subjects
Prior/concomitant medications, therapies, and medical/surgical interventions[v]	•	•	•	•	•	•	•	•	•	•	•	•	•	•

ADA=antidrug antibody; AE=adverse event; β-hCG=beta-human chorionic gonadotropin; cMRI=cardiac magnetic resonance imaging; ECG=electrocardiography; eGFR=estimated glomerular filtration rate; EOI=end of infusion; EOT=end of treatment; EOW=every other week; GLA=α-galactosidase A; HCV=hepatitis C virus; IP=investigational product; IRR=infusion-related reaction; lyso-Gb3=globotriaosylsphingosine; SAE=serious adverse event

[a] Screening procedures can be performed over multiple days within a period of up to 42 days following the subject's signature of the informed consent form. The value obtained for cMRI at Screening will serve as the baseline value. Subjects who screen failed or could not be enrolled during the screening period, due to circumstances including, but not limited to, COVID-19 related issues, may be rescreened with prior approval from the sponsor medical monitor. Some procedures, such as cMRI, may not need to be repeated for rescreening, with prior agreement of the sponsor medical monitor.

[b] All baseline assessments and procedures are to be performed at the clinical site. The baseline assessments may be completed over a period of 7 days as long as all baseline assessments and procedures are completed prior to the first REPLAGAL infusion. The day of the first REPLAGAL infusion is considered Day 0/Week 0 and all future study visits and windows should be timed from the first infusion.

[c] During the treatment period all study assessments and procedures should be completed on the day of the scheduled visit (ie, day of infusion) with the exception of visits where a cMRI will be performed (Screening, Week 52, and Week 104 Visits). On visits where a cMRI assessment will be performed, all study assessments should occur on the day of infusion; however, to accommodate the scheduling of cMRI, this assessment may be performed within ±7 days of the infusion day for Week 52 and ±14 days of the infusion day for Week 104.

[d] The first 3 REPLAGAL infusions (Day 0/Week 0 first infusion, EOW second infusion, and EOW third infusion) are to be administered at the clinical site. After the first 3 infusions, subjects who have not experienced a treatment-related serious adverse event (SAE) or a moderate or severe infusion-related reaction (IRR) may receive their subsequent infusions at home by qualified and trained medical personnel, per the discretion and direction of the investigator.
If at any point during home infusions subjects experience a treatment-related SAE or a moderate or severe IRR, their next infusion should be administered at the clinical site. Infusions should continue at the clinical site until no further moderate or severe IRRs are reported, at which point subjects may be re-evaluated for consideration to retransition to home infusions.

[e] Procedures and evaluations to be performed at site visits will occur at the clinical site.

[f] The safety follow-up period is 14 days (-4 days/+7 days) following end of treatment (EOT) infusion (or the last infusion for early discontinued subjects). At the end of this period there will be a telephone call initiated by the site staff to query for adverse events (AEs), SAEs and concomitant medications. Subjects who experienced SAEs, or

Table 1 Schedule of Activities

Study Assessments and Procedures	Screening Period[a]	Baseline Period[b]	Treatment Period[c]										Safety Follow-up Period
			EOW REPLAGAL Infusion[d] (±7 days) at (Week 2 -Week 50) and (±14 days) at (Week 52-Week 104)	Site Visits[e]									Telephone Contact/Visit[f]
	Day -50 to Day -8 (up to 42 days)	Day -7 to Day 0 (Day 0 = Week 0)[d]		Week 2 (±7 days)	Week 4 (±7 days)	Week 12 (±7 days)	Week 26 (±7 days)	Week 40 (±7 days)	Week 52 (±14 days)	Week 64 (±14 days)	Week 78 (±14 days)	Week 92 (±14 days)	Week 104 (±14 days) EOT or Early Termination[g]	14 Days (-4 days/ +7 days) from EOT or Last Infusion for Discontinued Subjects

mild-worsening, moderate or severe AEs will be requested to report to the clinical site for further examination. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure.

[g] For subjects who discontinue the study early, all ET assessments and procedures should be completed as soon as possible after the subject's last dose. For subjects who discontinue study treatment but remain in the study, the End of Treatment (EOT) Visit should occur within 10-21 days after the subject's last IP dose. These subjects will also have either a Week 104 Visit (if completing the study) or an ET Visit (if withdrawing from the study prior to Week 104).

[h] Informed consent must be obtained prior to performing any study related procedures.

[i] Fabry disease diagnosis will be confirmed at screening using a dried blood assay. For male subjects, Fabry disease will be confirmed by a deficiency of α-galactosidase A (GLA) activity and a mutation in the GLA gene. For female subjects, Fabry disease will be confirmed by a mutation in the GLA gene. Measurement of serum GLA activity will also be done for female subjects however, serum GLA activity is not an inclusion criterion for female subjects.

[j] The following vital signs will be collected at all study visits (screening and infusion visits): pulse, blood pressure, respiratory rate, and temperature. On infusion visits (baseline, EOW, and the site visits), vital signs should be obtained as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur on a visit, the vital signs collected within 10 minutes prior to the start of the expected infusion should be documented in the electronic case report form. If no infusion-related reactions (IRRs) are observed during the first 3 infusions, the observation and vital signs post infusion can be omitted at the discretion of the investigator.

[k] Subject height and weight must be measured and collected at Screening, Week 52 and Week 104 (or early termination) visits for all subjects.

[l] The first dose of REPLAGAL will be based on the subject's weight *at Baseline*. Change in subject weight of ≥±5% from baseline, or the last weight used to recalculate the dose, will require a recalculation of the dose by the clinical site. Weight will be measured approximately every 12 to 14 weeks, with any dosing adjustments completed prior to the next dose.

[m] REPLAGAL will be administered as an intravenous infusion over 40 minutes (±10 minutes) every other week (EOW) (±7 days) starting at the baseline visit up to Week 52 when EOW visits will have windows of ±14 days. All study procedures, assessments (except for postinfusion vital signs), and sample collection should be completed prior to administering an infusion.

[n] If the eGFR measurement at screening is not within the range, a second eGFR measurement may be completed and, if in range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first measurement.

[o] Female subjects of childbearing potential will have a pregnancy test administered at each study visit. At screening, pregnancy testing will be performed using a urine test and a serum human beta-human chorionic gonadotropin (β-hCG) test. The urine pregnancy test should be administered by the clinical site before any other screening procedures (except for signature of informed consent) are completed. If the urine pregnancy test is negative, screening procedures (including the serum pregnancy test) will be completed. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. No additional screening procedures should be completed until the result of the serum pregnancy test is available. Female subjects of childbearing potential must have a negative β-hCG serum test at screening to be eligible for the study. A positive β-hCG serum test would result in the subject being a screen failure. At baseline, the urine pregnancy test should be administered by the clinical

Table 1 Schedule of Activities

Study Assessments and Procedures	Screening Period[a]	Baseline Period[b]	Treatment Period[c]										Safety Follow-up Period
			EOW REPLAGAL Infusion[d] (±7 days) at (Week 2 -Week 50) and (±14 days) at (Week 52-Week 104)	Site Visits[e]									Telephone Contact/Visit[f]
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site before any other baseline procedures are completed. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. No additional baseline procedures will be completed until the result of the serum pregnancy test is available. If the serum β-hCG result is positive, the subject will not be dosed with REPLAGAL and would be considered a screen failure. At all other visits during the treatment period (EOW and site visits), a urine pregnancy test will be performed prior to the completion of any study procedures.

If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. The subject will not receive any additional REPLAGAL infusions until the result of the serum pregnancy test is available. If the serum β-hCG result is positive, no additional doses of REPLAGAL are to be administered.

[p] Serum chemistry laboratory tests: sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate, calcium, magnesium (Mg^{2+}), phosphate, creatinine, urea nitrogen, glucose, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, triglycerides, cholesterol, LDL cholesterol, and HDL cholesterol.

[q] Hematology laboratory tests include complete blood count: hematocrit [Hct]; hemoglobin [Hb]; RBC; WBC; platelets; basophils; basophils/total cells; eosinophils; eosinophils/total cells; immature granulocyte/leukocytes; lymphocytes; lymphocytes/total cells; monocytes; monocytes/total cells; neutrophils; and neutrophils/total cells.

[r] Serum inflammatory biomarkers include but are not limited to Interleukin 1 Beta, Tumor Necrosis Factor, Vascular Cell Adhesion Molecule 1, and Intercellular Adhesion Molecule 1.

[s] Antidrug antibody (ADA) samples to be collected prior to infusion; any samples that are confirmed positive for ADA will also be tested for neutralizing antibodies (NAbs).

[t] Proteinuria (measured by protein creatinine ratio) to be performed as part of urinalysis.

[u] Hepatitis B surface antigen, Hepatitis B core antibody, Hepatitis C (HCV) antibody with confirmation by HCV-ribonucleic acid polymerase chain reaction testing, human immunodeficiency virus antibody.

[v] The following medications and treatments are prohibited at any time prior and throughout the course of the study: FABRAZYME® (agalsidase beta) and its biosimilars, GLYSET® (miglitol), ZAVESCA® (miglustat), CERDELGA® (eliglustat), GALAFOLD® (migalastat), and any investigational product for treatment of Fabry disease. Treatment at any time during the study with the following medications is prohibited: Chloroquine, Amiodarone, Monobenzone, and Gentamicin.

2 INTRODUCTION

2.1 Indication and Current Treatment Options

Indication

Fabry disease is a rare, lifelong, slowly progressive, X-linked glycosphingolipid storage disorder with a global incidence estimated at 1:50,000 male live births (Spada et al. 2006). According to the census bureau's population, there are an estimated 11,000 patients with Fabry disease in the US.

Fabry disease results from a mutation in the α-galactosidase A gene located on chromosome Xq22.1, which leads to a partial or full loss of the activity of the lysosomal enzyme α-galactosidase A (GLA) (Garman and Garboczi 2004). More than 400 mutations have been identified in the GLA gene. These consist mainly of missense mutations, but nonsense mutations in addition to single amino acid deletions and insertions were also reported (Mehta et al. 2010). Most of these mutations are "private", having been identified only in individual families (Mehta et al. 2010). As an X-linked disorder, females may inherit an X-linked Fabry mutation from either parent accounting for twice the number of carriers (heterozygotes) as males (hemizygotes). Female carriers are not necessarily asymptomatic.

Alpha-galactosidase A is a lysosomal hydrolase enzyme that catalyzes the removal of terminal alpha-galactosyl moieties during the catabolism of glycolipids and glycoproteins. Partially active or deficient GLA results in abnormal accumulation of glycolipids (globotriaosylceramide [Gb_3]) in cells throughout the body, including capillary endothelial, renal (podocytes, tubular cells, glomerular endothelial, mesangial, and interstitial cells), cardiac (cardiomyocytes and fibroblasts) and nerve cells. Abnormal Gb_3 concentrations in membranous structures may impair intracellular trafficking processes, cellular activation, intercellular communication, and cellular homeostasis. This results in inflammatory changes and cytokine release, ultimately leading to apoptosis and eventual organ dysfunction and/or failure (Brady 1967; Desnick 1995; deVeber et al. 1992; Kahn 1973; Kaye et al. 1988; Schaefer et al. 2009).

Fabry disease is characterized by a range of phenotypes that generally correlates with the degree of residual enzymatic activity. The threshold level of alpha-Gal A activity below which clinically significant Fabry disease occurs is thought to be 30 to 35 percent of the mean normal control (Schiffmann et al. 2016). Phenotype presentation becomes more evident during childhood and young adulthood, and as the patient advances in age organ function deterioration intensifies ultimately leading to organ failure and early death. Most patients display multiple clinical manifestations of the disease, many of which may occur before 10 years of age in males (Deegan et al. 2006). In females, alpha-Gal A activity is most often higher than in males (Arends et al. 2017) and the onset of symptoms and time of diagnosis can be variable, occurring approximately 10 years later compared with males (Eng et al. 2007; Kruger et al. 2010). The clinical presentation of the disease varies widely among patients, but neuropathic pain and acroparesthesia are typically the first and most frequent symptoms to manifest.

Other clinical manifestations of Fabry disease include angiokeratomas, gastrointestinal problems, proteinuria, progressive renal impairment leading to renal failure, hypertrophic cardiomyopathy with arrhythmias, corneal dystrophy, hypohidrosis, and microvascular cerebral events including transient ischemic attacks, stroke, and dolichoectasia (Deegan et al. 2006; Schiffmann and Ries 2005). Vital organs, especially the heart, kidneys, and brain, are progressively affected with advancing age (Ries and Schiffmann 2005). As a consequence, physical and functional well-being and quality of life (QoL) are significantly reduced in patients with Fabry disease compared with the general population and patients with other chronic nongenetic disorders (Gold et al. 2002; Miners et al. 2002).

Patients with Fabry disease have a shortened life expectancy with men and women affected by the disorder living 20 years (MacDermot et al. 2001b) and 15 years (Barbey et al. 2004; MacDermot et al. 2001a; Mehta et al. 2004) less than those in the general population, respectively. Prior to the introduction of enzyme replacement therapy (ERT) in 2001, the leading cause of death in patients with Fabry disease was renal disease (Mehta et al. 2009). Since then, significant improvements have been made in supportive care of renal function and cardiac disease is now the primary cause of death (Mehta et al. 2009; Waldek et al. 2009).

Current Treatment Options

Two ERT products have been approved for patients with a confirmed diagnosis of Fabry disease: agalsidase alfa (marketed under the trade name of REPLAGAL® in countries outside the United States [US] by Shire) and agalsidase beta (FABRAZYME; Genzyme). REPLAGAL is approved in 59 countries worldwide as of December 2017, including Canada. More recently in May 2016, migalastat (GALAFOLD) was granted approval in Europe and the US as the first chaperone therapy for the long-term treatment of Fabry disease in adults and adolescents aged 16 or older with an amenable mutation in GLA.

2.2 Product Background, Clinical Information, and Risk Benefit

Product Background

Always refer to the latest version of the REPLAGAL investigator's brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of REPLAGAL.

Alpha-galactosidase A is a homodimer consisting of 2 approximately 50 kDa subunits; α-galactosidase A is targeted to its lysosomal site of action by mannose-6-phosphate (M6P) moieties, which bind to their cognate receptors in the Golgi directing the enzyme to the prelysosomal compartments. Any enzyme that escapes this routing system is secreted by the cell via the constitutive secretory pathway and is often recaptured by cell surface M6P receptors to be rerouted to the lysosome by the endocytic pathway (Kornfeld and Mellman 1989). The M6P-mediated lysosomal targeting is the key mechanism that made ERT a feasible treatment strategy for lysosomal storage diseases as it enables the targeting of exogenously administered lysosomal enzymes to the appropriate subcellular compartment for their optimal activity.

REPLAGAL (agalsidase alfa) is human GLA produced by genetic engineering technology. REPLAGAL is manufactured using an aseptic filling process in a facility in compliance with current Good Manufacturing Practice and regulations. REPLAGAL is formulated as a sterile product suitable for parenteral administration. The formulation of REPLAGAL includes sodium phosphate as a buffering agent, polysorbate 20 as a stabilizing agent, and sodium chloride as an isotonic agent.

REPLAGAL is intended for use as an ERT for patients with Fabry disease. Replacement of the absent or deficient enzyme contributes to the correction of the deficient enzymatic activity and allows for improved metabolism of the natural substrate of the enzyme. Over time, chronic replacement of the deficient enzyme could theoretically alter the natural history of Fabry disease, with concomitant improvements in renal and cardiac function, metabolism, neuropathic pain, and QoL.

Clinical Information and Benefit/Risk

Clinical studies of REPLAGAL have been conducted in Australia, Brazil, Canada, Europe, Japan, Paraguay, and the US. The clinical development program was designed to evaluate the safety and efficacy of REPLAGAL in subjects with Fabry disease and includes early phase safety studies, placebo-controlled safety and efficacy studies, and open-label extension studies. Additionally, studies in special populations have been conducted: pediatric subjects, female subjects, dialysis and renal-transplant subjects, and 1 compassionate-use study. A manufacturing change led to additional studies, such as bioequivalence pharmacokinetic (PK)/pharmacodynamic (PD) studies. One open-label, treatment protocol study (HGT-REP-081) evaluating the long-term safety of REPLAGAL in subjects with Fabry disease following a manufacturing change from roller bottle (RB) to bioreactor, animal-free (AF) process was completed in Canada (2018).

Data collected in clinical studies to date indicate that REPLAGAL remains safe and well-tolerated during long-term use. As with other intravenous (IV)-administered protein therapeutics, the identified and potential risks of treatment with REPLAGAL include occurrence of infusion-related reactions (IRRs) and potential development of neutralizing antibodies (NAbs) that may compromise efficacy. IRRs are idiosyncratic allergic-type hypersensitivity signs or symptoms experienced by patients during the infusion of REPLAGAL or on the first day of its administration. Infusion-related reactions are the most commonly observed ADRs associated with REPLAGAL. The onset of IRRs generally occurs within the first 2 to 4 months after initiation of treatment with REPLAGAL, although later onset (after 1 year) has been reported. The frequency of these events has decreased over time as the number of infusions increased. The clinical and commercial experience has indicated that IRRs can be mitigated with a combination of infusion-rate control and pretreatment with medications such as antihistamines, antipyretics or low-dose corticosteroids. Cardiac ischemic events and arrhythmias triggered by IRRs have been also reported as risks associated with REPLAGAL treatment in patients with pre-existing cardiac manifestations of Fabry disease. The majority of these events were mild in severity, nonserious and resolved. Cardiac events triggered by IRRs are possibly due to the hemodynamic stress caused by infusion. Lack of efficacy due to development of NAbs against agalsidase alfa remains a potential risk but has not been reported during treatment with REPLAGAL.

To date, there is no apparent evidence for a loss of efficacy due to NAbs. Borderline immunoglobulins E (IgE) antibody positivity not associated with anaphylaxis has been reported in clinical studies. Additionally, clinical and postmarketing experience has reflected a safety profile for REPLAGAL that continues to support administration in the home setting.

In summary, the cumulative clinical and postmarketing experience indicates that the benefit-risk profile of agalsidase alfa for the treatment of Fabry disease remains favorable supported by the following:

- A high disease burden with life-threatening/debilitating complications
- Alternative treatments are limited and are also associated with significant adverse reactions
- Demonstrated benefit in pain reduction, improvement or stabilization of cardiac and renal function, and reduction in accumulation of Gb_3
- Well established safety profile with the main identified risks of IRRs, cardiac events triggered by IRRs, and immunogenicity.

The efficacy profile of REPLAGAL was evaluated in clinical studies and REPLAGAL received initial regulatory approval in the European Union (EU) in 2001 based on reduction of pain in patients with Fabry disease. Additionally, the registration studies evaluated important clinical outcome benefits such as QoL, renal function, cardiac structure, and PD (Gb_3) measures.

The benefit-risk for this study is in alignment with the overall clinical program benefit-risk assessment. Investigators will follow site-specific standards for the mitigation of impact from COVID-19 and will manage any AEs related to COVID-19 per study procedures for the reporting and treatment of AEs.

2.3 Study Rationale

Initial approval for REPLAGAL was granted in Canada in 2004. At that time, REPLAGAL was manufactured using an RB technology. In March 2009, Shire submitted a Supplemental New Drug Submission for REPLAGAL manufactured using a new bioreactor method intended to eliminate animal-sourced raw materials from the manufacturing process but approval was not granted. On 10 Feb 2017, REPLAGAL manufactured using the AF process received conditional approval in Canada under a Notice of Compliance with Conditions mandating that efficacy of REPLAGAL be confirmed in patients with Fabry disease. This Phase 3 study will be conducted to fulfill the postmarketing commitment to confirm the efficacy of REPLAGAL in a population of treatment-naïve subjects with a confirmed diagnosis of Fabry disease.

2.4 Compliance Statement

This study will be conducted in accordance with this protocol, the International Council for Harmonisation Guideline for Good Clinical Practice E6 (ICH GCP, 1996; E6 R2, 2017), Title 21 of the US Code of Federal Regulations (US CFR), the EU Directives (2001/20/EC; 2005/28/EC), and applicable national and local regulatory requirements.

The responsibilities of the study sponsor and investigator(s) are described fully in Appendix 1.

3 OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objective

The primary objective is to evaluate the efficacy of REPLAGAL (0.2 mg/kg every other week [EOW] up to 104 weeks) on renal (estimated glomerular filtration rate [eGFR]) and cardiac (left ventricular mass index [LVMI]) parameters in subjects with a confirmed diagnosis of Fabry disease and who are naïve to any Fabry-specific treatment.

3.1.2 Secondary Objectives

The secondary objectives of this study are:

- To evaluate the efficacy of REPLAGAL 0.2 mg/kg EOW on other renal and cardiac variables and PD markers
- To assess the safety and tolerability of REPLAGAL over the study period.

3.1.3 Exploratory Objectives

The exploratory objectives of this study are:

- To evaluate the effect of REPLAGAL on inflammatory biomarkers relevant to Fabry disease
- To assess the effect of REPLAGAL on the subject's overall rating of pain, fatigue, severity of Fabry symptoms and quality of life
- To evaluate the effects of REPLAGAL on gastrointestinal (GI) symptoms of Fabry disease.

3.1.4 Rationale for the Selection of the Primary Endpoints

A number of clinical and PD measures have been evaluated as endpoints in previous clinical studies of Fabry disease, including renal function, cardiac involvement, pain, Gb_3 and lyso-Gb^3 levels and occurrence of sentinel events (renal, cardiac, or cerebrovascular events, or death). Sentinel events, such as hospitalizations and death events are infrequent, often unpredictable (eg, arrhythmias, stroke) and occur over a protracted period during the progressive course of Fabry disease and therefore precludes their use as practical endpoints for disease progression in a clinical study setting.

Measures of renal and cardiac involvement have been more consistently used to assess disease progression across clinical studies based on the progressive impairment of the kidney and the heart during the disease evolution (Branton et al. 2002; Kampmann et al. 2008; Schaefer et al. 2009; Schiffmann et al. 2009). Over time, progressive deterioration of renal and cardiac function results in the occurrence of clinical events such as myocardial infarction, arrhythmia, dialysis, left ventricular hypertrophy, and end-stage renal disease (dialysis and renal transplantation), and may finally lead to death related to cardiac or renal failures.

The clinical relevance of renal and cardiac parameters, as disease progression markers, is highlighted by their inclusion in global treatment guidelines and in the Canadian Fabry Disease Treatment Guidelines 2017, as key criteria informing initiation of enzyme replacement therapy (ERT) for the stabilization or improvement of renal and cardiac function decline (Eng et al. 2006; Schiffmann et al. 2010; Sirrs et al. 2018; West et al. 2012). Based on the current literature, the Canadian Fabry Disease Treatment Guidelines 2017 confirmed that there is evidence of improvement with ERT as disease specific therapy, as follows:

- Stabilization of Fabry nephropathy with stable proteinuria and glomerular filtration rate
- Stabilization of Fabry cardiomyopathy with stable or declining left ventricular mass index or left ventricular wall thickness.

Renal and cardiac disease manifestations are also considered as the primary criteria for ERT initiation in patients with Fabry disease and are assigned a higher level of evidence to justify ERT treatment relative to neurologic, neuropathic, and gastrointestinal manifestations. In this sense, the renal disease evidence has the level of evidence 1 - Grade B, while the cardiac disease evidence has the level of evidence 2 - Grade B (Sirrs et al. 2018).

Considering the clinical relevance of renal and cardiac involvement in Fabry disease, it is appropriate to look at measures of renal and cardiac function as relevant clinical endpoints when assessing the efficacy of Fabry disease treatments. The same renal and cardiac parameters were also used as primary endpoints by other sponsors for studying the efficacy of their molecules on Fabry disease (eg, FABRAZYME®, Genzyme [(Fabrazyme 2003)]; GALAFOLD®, Amicus [(Galafold 2018)]; and pegunigalsidase alfa [PRX-102], Protalix).

3.2 Study Endpoints

The study endpoints are summarized and mapped to their corresponding objective in Table 2. A detailed description of the statistical analysis of each endpoint is provided in Section 8.2.6.

Table 2 Objectives and Endpoints

Objective	Endpoint(s)
Primary	
To evaluate the efficacy of REPLAGAL (0.2 mg/kg every EOW up to 104 weeks) on renal (eGFR) and cardiac (LVMI) parameters in subjects with a confirmed diagnosis of Fabry disease and who are naïve to any Fabry-specific treatment	• Change from baseline at Week 104 in renal function, assessed by eGFR (using the Chronic Kidney Disease Epidemiology [CKD-EPI] formula) • Change from baseline at Week 104 in cardiac structure, assessed by LVMI using cardiac magnetic resonance imaging (cMRI).
Secondary	
To evaluate the efficacy of REPLAGAL 0.2 mg/kg EOW on other renal and cardiac variables and PD markers.	• Annualized rate of change in eGFR • Annualized rate of change in LVMI • Change over time in eGFR • Change over time in LVMI • Change over time in proteinuria, measured by protein/creatinine ratio (PCR) • Change over time of the number of fibrotic segments suggestive of cardiac fibrosis as assessed by volume of fibrosis, measured by cMRI • Change over time in interventricular septal end-diastolic thickness (IVSTd) and posterior wall thickness in diastole (PWTd), measured by cMRI • Change over time in plasma globotriaosylsphingosine (lyso-Gb3)
To assess the safety and tolerability of REPLAGAL over the study period.	• Adverse events (AEs) • Antidrug antibody (ADA) assessments including NAbs • Clinical laboratory tests (serum chemistry, hematology, and urinalysis) • Electrocardiography • Vital signs

Table 2 Objectives and Endpoints

Objective	Endpoint(s)
Exploratory	
To evaluate the effect of REPLAGAL on inflammatory biomarkers relevant to Fabry disease.	• Change over time in serum and levels of inflammatory biomarkers, including but not limited to Interleukin 1 Beta (INTLK1β), Tumor Necrosis Factor (TNF-α), Vascular Cell Adhesion Molecule 1 (VCAM1), and Intercellular Adhesion Molecule 1 (ICAM1).
To assess the effect of REPLAGAL on the subject's overall rating of pain, fatigue, severity of Fabry symptoms and quality of life.	• Change over time in the following patient-reported outcome (PRO) measures: o Patient-reported pain assessed by the Brief Pain Inventory-Short Form (BPI-SF) o Patient-reported fatigue assessed by the Brief Fatigue Inventory (BFI) o Patient-reported disease severity assessed by the Patient Global Impression of Fabry Symptom Severity (PGI-S) o Patient-reported QoL assessed by the Short Form-36 Health Survey (SF-36v2)
To evaluate the effects of REPLAGAL on gastrointestinal symptoms of Fabry disease.	• Change over time in GI symptoms as assessed by the Gastrointestinal Symptom Rating Scale (GSRS)

ADA=antidrug antibody; AE=adverse event; AUC=area under the plasma concentration-time curve; BFI=Brief Fatigue Inventory; BPI-SF=Brief Pain Inventory-Short Form; CKD-EPI=Chronic Kidney Disease-Epidemiology; cMRI=cardiac magnetic resonance imaging; eGFR=estimated glomerular filtration rate; EOW=every-other-week; GSRS=Gastrointestinal Symptom Rating Scale; ICAM1=Intercellular Adhesion Molecule 1; INTLK1β =Interleukin 1 Beta; $IVST_d$=interventricular septal end-diastolic thickness; LVMI=left ventricular mass index; lyso-Gb3=globotriaosylsphingosine; NAb=neutralizing antibody; PCR=protein/creatinine ratio; PD=pharmacodynamic; PGI-S=Patient Global Impression of Fabry Symptom Severity; PRO=patient-reported outcome; PWTd=posterior wall thickness in diastole; QoL=quality of life; SF-36v2=Short Form-36 Health Survey; TNF-α=Tumor Necrosis Factor; VCAM1=Vascular Cell Adhesion Molecule 1

4 STUDY DESIGN

4.1 Overall Design

This is a global, Phase 3, multicenter, nonrandomized, open-label, single-arm, and baseline-controlled study.

Eligible participants will be male and female adult subjects who are treatment naïve and who have a confirmed diagnosis of Fabry disease with left ventricular hypertrophy. Subjects with or without renal involvement may qualify for the study if their eGFR is 45 to 120 mL/min/1.73 m^2, as calculated by the Chronic Kidney Disease-Epidemiology (CKD-EPI) formula.

The study will include 1 arm, comprising subjects prospectively treated with REPLAGAL for 104 weeks. The change from baseline to Week 104 in eGFR and LVMI will be compared with values of patients with Fabry disease from the published literature (Schiffmann et al. 2009); (Kampmann et al. 2008) who had similar baseline demographic and disease characteristics as the study subjects (Schiffmann et al. 2009) and who remained untreated for 2 years.

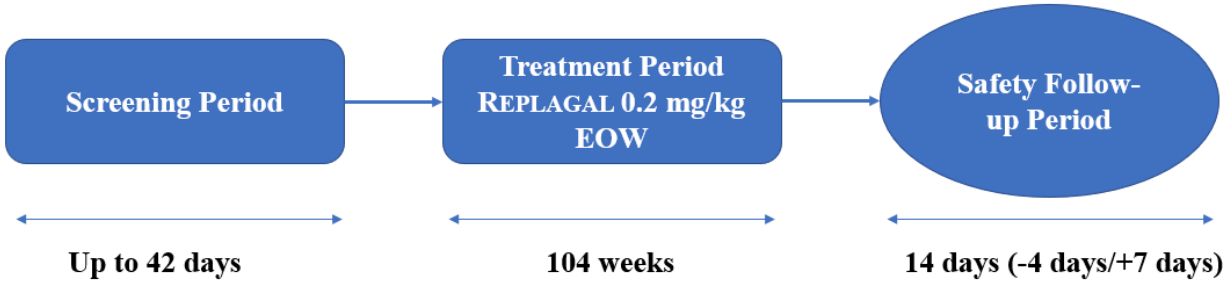
It is expected that at least 45 adult subjects with Fabry disease will need to be enrolled in order to obtain at least 36 evaluable subjects. To be considered evaluable, a subject must not have discontinued from investigational product and must have valid, nonmissing values of the primary efficacy endpoints (eGFR and LVMI) for both baseline and Week 104.

Potential subjects will participate in a screening period, which can last up to 42 days, to determine eligibility. After completion of baseline procedures and assessments, eligible subjects will receive 0.2 mg/kg REPLAGAL by IV infusion EOW for 104 weeks and will have study visits approximately every 12 to 14 weeks during the 104-week treatment period. Subjects who discontinue from study treatment may continue to have study visits if they consent to do so. Approximately 14 days (-4 days/+7 days) after the last infusion or study visit, a safety follow-up contact or visit will occur to ensure the subject's safety.

Subject participation is expected to be approximately 112 weeks, as follows (Figure 1):

- Screening period: up to 42 days
- Treatment period: 104 weeks
- Safety follow-up period: 14 days (-4 days/+7 days).

Figure 1 Study Design Flow Chart



EOW=every other week

4.2 Scientific Rationale for the Reference-Controlled Study Design

This study intends to confirm the efficacy of REPLAGAL in treatment-naïve subjects who have a confirmed diagnosis of Fabry disease. These criteria pose a challenge for subject recruitment, precluding the feasibility of a randomized placebo-controlled design recommended for Phase 3 studies as detailed below.

Fabry disease is a rare disease and recruitment of sufficient number of subjects to conduct clinical studies has been a longstanding challenge throughout the clinical development of REPLAGAL. Following approval of ERT for Fabry disease (REPLAGAL and Fabrazyme) in 2001, treatment was indicated for patients with more advanced disease manifestations and subsequent treatment guidelines encouraged the initiation of treatment at the time of diagnosis in males and at the time of development of significant symptoms in females (Schiffmann et al. 2010); (Eng et al. 2006; Sirrs et al. 2018; West et al. 2012). As such, patients with Fabry disease who remain naïve to treatment in countries where ERT is commercially available are typically those who do not show evidence of severe organ involvement. The rarity of Fabry disease and the recommended guidelines for the management of its symptoms limit the availability of the clinical population targeted for this study, posing challenges for the recruitment of a sufficient number of naïve subjects.

The Canadian Fabry Disease Treatment Guidelines (Sirrs et al. 2018) outline when patients qualify for disease specific therapy based on available data and recommend regular follow-up of patients who do not yet meet the criteria for treatment (Sirrs et al. 2018). According to the guidelines, patients with renal and cardiac manifestations are all eligible for treatment, which limits the number of available ERT-naïve patients and has implications on the study design. The small global pool of patients who are potentially eligible for this study limits the feasibility of an adequately powered 2-arm, randomized, placebo-controlled phase 3 study, to detect between-arm differences in the primary endpoints. Due to the recruitment and ethical limitations associated with the 2-arm, placebo-controlled study design, the focus has shifted towards a design whereby the efficacy of REPLAGAL will be confirmed by comparing the dataset from the prospectively treated cohort enrolled in this study with an external, reference dataset of untreated patients (FDA Guidance for Industry: E10 Choice of Control Group and Related Issues in Clinical Trials).

More specifically, eGFR and LVMI change over the 2-year treatment period will be compared with reported literature values for patients with Fabry disease who remained untreated for 2 years and who demonstrate similar baseline demographic and disease severity characteristics as those in the treatment arm.

Study designs relying on comparisons with reference datasets from untreated patients have become increasingly common for studies aiming to investigate the benefits of ERT for the treatment of Fabry disease. Except for one long-term, placebo-controlled Phase IV study conducted with agalsidase beta (Banikazemi et al. 2007), none of the long-term studies performed after the approval and marketing of ERT have used a concurrent, untreated study arm. Access to ERT treatment has changed the treatment paradigm for patients with Fabry disease and limited the availability of data in treatment-naïve patients to the timeframe before approval of ERTs. Summaries of selected studies examining the effects of ERT in patients with Fabry disease by comparing outcomes from a treated cohort with reference data from untreated patients are provided below to support the validity of this approach. For each study summary, the focus is on the reasons justifying the use of a reference dataset from untreated patients as a comparator and on the criteria used for patient selection to ensure that the treated and the untreated patients have similar baseline characteristics to allow for a valid comparison.

- **Rombach et al., 2013:** this prospective Dutch study sought to examine the long-term benefits of ERT (Rombach et al. 2013). Renal, cardiac, and cerebrovascular measures were collected from 75 ERT-treated patients with Fabry disease between 1999 and 2010 and compared with data from a natural history cohort. The natural history cohort data were obtained from the medical records of 28 patients with Fabry disease who had a history of complications before ERT became available or who had an indication for ERT but remained untreated after treatment became available. Among the untreated patients, only those meeting the Dutch Fabry Guidelines criteria for treatment defined as the presence of chronic kidney disease (CKD), left ventricular hypertrophy, or cerebral white matter lesions were selected. The selection criteria set for the historical control were intended to create an untreated cohort of patients who matched those in the treatment arm by disease severity, in order to prevent confounding result interpretation due to bias by indication (Rombach et al. 2013).

- **Weidemann et al., 2013:** this study conducted in Germany aimed to examine the effects of ERT on the progression towards "hard" clinical endpoints including stroke, end-stage renal disease and initiation of dialysis, and Fabry disease-related death in patients with Fabry disease (Weidemann et al. 2013). The treatment cohort data was collected from 40 patients with Fabry disease registered in the Wurzburg Fabry disease Centre (established in 2001) who have been receiving ERT for at least 5 years (or died during the observation period). Patients in treatment were relatively old with advanced disease progression at baseline because ERT was not available prior 2001. The authors noted that after approval of ERT, it was inappropriate to conduct a prospective study enrolling untreated subjects for ethical considerations. Therefore, historical, registry data from untreated subjects were used for comparison.
  Forty patients from the Fabry Registry, a database that monitors the natural history of

Fabry disease and outcomes of patients, were selected to match those in the treatment group with respect to age, gender, previous transient ischemic attacks, and CKD stage. These patients remained untreated due to ERT reimbursement issues in their countries of residence (Weidemann et al. 2013).

- **Beck et al., 2015:** this retrospective study was conducted to investigate the long-term benefits of ERT treatment on renal function, cardiomyopathy, morbidity and mortality in patients with Fabry disease (Beck et al. 2015). The outcomes evaluated were annualized rate of change in eGFR and LVMI, as well as time to and age at composite morbidity endpoints and death. Data for the treated cohort was collected from 740 patients enrolled in the Fabry Outcome Survey (FOS) registry. The authors highlighted that selection of an untreated cohort from FOS patients resulted in a biased comparison group, as untreated patients within the registry tend to be less affected by the disease than those receiving treatment and were predominantly females. Only 10% of FOS untreated patients could be matched for comparison resulting in a sample too small to allow for valid conclusions. As a result, data from FOS were extracted to provide cohorts of treated patients as comparable as possible to untreated cohorts from published studies with respect to inclusion/exclusion criteria and available data. Data from the FOS evaluable treated renal cohort were compared with those from untreated patients reported in a natural history study by Schiffman et al. (Schiffmann et al. 2009) as the two groups were broadly comparable in terms of age and renal characteristics. The FOS evaluable treated cardiac cohort was compared with an untreated cohort of patient with Fabry disease from a natural history study by Kampmann et al. (Kampmann et al. 2008), as the two cohorts were closely similar in terms of patient number, age, and relevant clinical factors.
Two untreated comparators from the literature were used to evaluate morbidity-related outcomes: one was a natural history cohort (Schiffmann et al. 2009) while the other was a placebo-treated cohort from a randomized clinical study of agalsidase beta (Banikazemi et al. 2007).

In the proposed SHP675-301 study design, each subject is their own control and the assessment will be made by comparing their postbaseline results with their own baseline results for eGFR and LVMI. To determine treatment benefits, the change in eGFR and LVMI in the prospectively treated cohort over the 2-year treatment period will be compared with reference control values of untreated patients with similar baseline demographic and disease characteristics from the published literature. While use of reference control values is not optimal, and elimination of bias in subject baseline characteristics is challenging, every effort to facilitate meaningful matches will be made. After a comprehensive review of published/available data, the following values from nontreated reference cohorts have been selected as comparators and will be used in the statistical analysis of the primary endpoints (refer to Section 9.5.1 for more details on the statistical analysis of the primary endpoints):

- For the value of the weighted annualized rate of change in eGFR from natural history patients (-3 mL/min/1.73 m^2) refer to analysis by Schiffmann et al. (Schiffmann et al. 2009)

- For the value of the weighted annualized rate of change in LVMI from natural history patients (+5 g/m$^{2.7}$) refer to analysis Kampmann et al. (Kampmann et al. 2008).

4.3 Justification for Dose

REPLAGAL 1 mg/mL concentrate solution for infusion is approved in 63 countries worldwide as of January 2020. The approved dose for REPLAGAL in these countries is 0.2 mg/kg of body weight EOW by IV infusion over 40 minutes (±10 minutes). This is the dose to be used for this study.

4.4 Duration of Subject Participation and Study Completion Definition

A subject's maximum duration of participation is approximately 112 weeks. This includes the screening period (up to 42 days), the treatment period (104 weeks), and the safety follow-up period (14 days [-4 days/+7 days] from the end of treatment [EOT] infusion, or the last infusion for subjects who withdraw early from the study). A subject is considered enrolled in the study when he or she signs informed consent and passes the inclusion/exclusion criteria.

The overall planned study duration is approximately 6 years. The first subject is expected to be enrolled in 2022. The Study Completion Date is defined as the date on which the last subject in the study completes the final protocol-defined assessment(s). This includes the follow-up visit or contact, whichever is later (refer to Section 8.1.4 for the defined follow-up period for this protocol).

4.5 Sites and Regions

This study is expected to be conducted at up to 50 sites globally.

5 STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent/assent before any procedures specified in the protocol are performed.

5.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below:

1. The subject must voluntarily sign an Institutional Review Board (IRB)/Independent Ethics Committee/Research Ethics Board approved informed consent form after all relevant aspects of the study have been explained and discussed with the subject.
2. The subject has Fabry disease as confirmed at screening by the following criteria using a dried blood spot (DBS) assay:
   - For male subjects, Fabry disease is confirmed by a deficiency of GLA activity and a mutation in the GLA gene.
   - For female subjects, Fabry disease is confirmed by a mutation in the GLA gene.
3. The subject is 18 to 65 years of age, inclusive.
4. Female subjects must have a negative pregnancy test at screening.
5. Female subjects of child-bearing potential must agree to use a medically acceptable method of contraception at all times during the study and for at least 14 days after the final study infusion; the methods of acceptable contraception are listed in the protocol.
6. The subject is deemed, as determined by the investigator, to have adequate general health to undergo the specified protocol-related procedures and to have no safety or medical contraindications for participation.
7. The subject has not received any treatment (approved or investigational) specific to Fabry disease, such as ERT, chaperone therapy, or substrate reduction therapy.
8. The subject must have an eGFR of 45 to 120 mL/min/1.73 m^2; eGFR will be calculated by a Shire-designated laboratory using the CKD-EPI formula. If the eGFR measurement at screening is not within the stipulated range, a second eGFR measurement may be completed and, if in range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first measurement. This inclusion criterion follows the European Guidelines for Treatment of Fabry Disease (Biegstraaten et al. 2015) and Kidney Disease Improving Global Outcomes guidelines for classification of renal disease (Kidney Disease Improving Global Outcomes (KDIGO) 2013).
9. The subject has left ventricular hypertrophy (LVH), where LVH is defined as left ventricular mass index (LVMI) >50 g/$m^{2.7}$ confirmed by cardiac magnetic resonance imaging (cMRI) at screening. The cMRI value at screening will serve as the baseline value.

5.2 Exclusion Criteria

The subject will be excluded from the study if any of the following exclusion criteria are met:

1. In the opinion of the investigator, the subject's life expectancy is ≤5 years.
2. The subject has undergone or is scheduled to undergo kidney transplantation or is currently on dialysis, or has any signs or symptoms of end stage renal disease.
3. Urine protein/creatinine ratio (PCR) >1.5 mg/mg.
4. Subjects who have clinically relevant history of allergy or signs or symptoms of severe hypersensitivity (including hypersensitivity to the REPLAGAL active substance or any of the excipients), which in the investigator's judgment, will substantially increase the subject's risk if he or she participates in the study.
5. Cardiac fibrosis involving more than 2 segments, as determined by cMRI at screening.
6. In the opinion of the investigator, the subject has non-Fabry disease-related cause of end-organ (renal, cardiac, central nervous system) dysfunction/failure or is receiving medications that may affect the rate of disease progression, as assessed by cardiac and/or renal measures.
7. The subject has a positive test at screening for hepatitis B surface antigen (HBsAg), positive test for hepatitis B core antibody (HBcAb), positive test for hepatitis C (HCV) antibody with confirmation by HCV-ribonucleic acid polymerase chain reaction testing, or positive test for human immunodeficiency virus (HIV) antibody.
8. Treatment with REPLAGAL at any time prior to the study.
9. Prior treatment with any of the following medications:
    - FABRAZYME (agalsidase beta) and its biosimilars
    - GLYSET® (MIGLITOL)
    - ZAVESCA® (MIGLUSTAT)
    - CERDELGA® (ELIGLUSTAT)
    - GALAFOLD® (MIGALASTAT)
    - ANY INVESTIGATIONAL PRODUCT FOR TREATMENT OF FABRY DISEASE
    - CHLOROQUINE
    - AMIODARONE
    - MONOBENZONE
    - GENTAMICIN.
10. The subject is pregnant or lactating.
11. The subject has a body mass index >39 kg/m^2. (BMI = kg/m^2)
12. The subject is treated or has been treated with any other investigational drug within 30 days ~~of~~ prior to study start.

13. The subject is unable to understand the nature, scope, and possible consequences of the study.
14. The subject is unable to comply with the protocol, eg, uncooperative with protocol schedule, refusal to agree to all of the study procedures, inability to return for evaluations, or is otherwise unlikely to complete the study, as determined by the investigator.

5.3 Restrictions

Not applicable.

5.4 Reproductive Potential

5.4.1 Female Contraception

Sexually active females of childbearing potential should be using an acceptable form of contraception. Females of childbearing potential must be advised to use acceptable contraceptives throughout the study period and for 14 days following the last dose of investigational product. If hormonal contraceptives are used, they should be administered according to the package insert. Females of childbearing potential who are not currently sexually active must agree to use acceptable contraception, as defined below, if they become sexually active during the period of the study and 14 days following the last dose of investigational product.

Female subjects should be either:

- Postmenopausal (12 consecutive months of spontaneous amenorrhea and age ≥51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post sterilization, or
- Females of child-bearing potential with a negative urine and/or serum beta-human chorionic gonadotropin (β-hCG) pregnancy test as described in Section 8.2.3.7. Females of childbearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception.

Acceptable methods of contraception are listed in Appendix 4.

5.4.2 Male Contraception

Not applicable.

6 STUDY INTERVENTION

6.1 Investigational Product

6.1.1 Identity of Investigational Product

The test product is REPLAGAL, which will be supplied as a sterile, clear, colorless concentrate for dilution for IV infusion. REPLAGAL will be provided in single-use, 5 mL vials containing 3.5 mL of concentrate at a concentration of 1 mg/mL.

Additional information is provided in the current REPLAGAL investigator's brochure.

6.1.2 Blinding the Treatment Assignment

Not applicable.

6.2 Administration of Investigational Product

REPLAGAL infusions may occur at the clinical site, in a home setting, or at a qualified satellite treatment center at the investigator's discretion. Subjects must complete all baseline assessments and procedures before the first infusion of REPLAGAL. Subjects will receive REPLAGAL at a dose of 0.2 mg/kg body weight administered as an IV infusion over 40 minutes (±10 minutes) EOW (±7 days). Refer to the instruction manual provided separately from this protocol that outlines all operating procedures to be followed for this study including drug transport, reconstitution, and the required subject assessments before, during, and after infusion of REPLAGAL.

6.2.1 Infusions of REPLAGAL in the Home Setting

Please note that allowing for subjects to have home infusions is not a study requirement. This is an option available to the investigator as per their discretion and/or in accordance with regulatory requirements per country.

For those sites choosing to allow subjects to dose in a home setting, the first 3 REPLAGAL infusions (baseline infusion [Day 0/Week 0], Week 2/EOW infusion 2, and Week 4/EOW infusion 3) are to be administered at the clinical site. After the first 3 infusions, subjects who have not experienced a treatment-related serious adverse event (SAE) or a moderate or severe IRR (refer to Appendix 3.1 for a definition of IRR) may have their subsequent EOW infusions administered by qualified and trained medical personnel at home, per the discretion and direction of the investigator. Subjects receiving REPLAGAL as home therapy will be required to return to the clinical site to receive their infusion at Week 12/EOW infusion 7, Week 26/EOW infusion 14, Week 40/EOW infusion 21, Week 52/EOW infusion 27, Week 64/EOW infusion 33, Week 78/EOW infusion 40, Week 92/EOW infusion 47, and Week 104 visits/EOW infusion 53. A total of 11 REPLAGAL infusions are required to be performed at the clinical site.

If at any point during home infusions subjects experience a treatment-related SAE or a moderate or severe IRR, their next infusion should be administered at the clinical site. Infusions should continue at the clinical site until no further moderate or severe IRRs are reported, at which point subjects may be re-evaluated for consideration to retransition to home infusions.

Home therapy can only resume at subsequent infusions after agreement is reached by the investigator and the Shire medical monitor.

In the home setting, vital signs and documentation of AEs will be collected at each visit. The qualified, trained medical personnel will evaluate and report to the study site the occurrence of AEs. Study site personnel will report SAEs as described in the Emergency Contact Information and Appendix 3.4. If a SAE or moderate or severe IRR occur while a subject is receiving treatment at home, the qualified, trained medical personnel will maintain contact with the investigator for treatment advice.

At each home visit, a urine pregnancy test will be conducted for women of childbearing potential prior to the completion of any study procedures. If the result is positive, the infusion will not be administered, and a blood sample will be collected for serum β-hCG pregnancy testing. The next infusion of REPLAGAL will not be administered until the results of the serum pregnancy test are received. If the serum β-hCG result is also positive, no additional doses of the investigational product are to be administered. Pregnancy should be reported to Shire Global Patient Safety Evaluation, and the Shire medical monitor (or designee) within 24 hours of becoming aware of the pregnancy. For more details on pregnancy reporting refer to Appendix 3.8.

If there are no IRRs during the first 3 infusions the observation and vital signs post infusion can be omitted at the discretion of the investigator.

The qualified, trained medical personnel will follow the instruction manual provided separately from this protocol that outlines all operating procedures to be followed for this study including drug transport, reconstitution, and the required subject assessments before, during, and after infusion of REPLAGAL. Clinical evaluations will remain under the medical supervision of the investigator. Appropriate medical support, including adequately trained personnel in emergency measures, should be readily available when REPLAGAL is administered. If anaphylactic or other acute severe reactions occur, immediately discontinue the infusion and initiate appropriate medical treatment.

6.2.2 Management of Infusion-related Reactions

For guidance on the management of IRRs, refer to Appendix 3.1.

6.2.3 Interactive Response Technology for Investigational Product Management

An interactive response technology (IRT) will be used for investigational product management tasks including investigational product supply management, inventory management and supply ordering, investigational product expiration tracking, destruction at site tracking, and return of investigational product. Please refer to the instruction manual provided separately from this protocol that outlines the operating procedures regarding the IRT.

At relevant visits during the baseline and treatment periods, patient-facing assessments including the SF-36, BPI-SF, PGI-S, BFI, and GSRS will be collected via an electronic device. These assessments will be captured and transmitted to a third-party database where site staff will have access to the patient data via a restricted website.

In the case of an unplanned event that disallows/prevents the administration of the PRO on a device, for reasons including but not limited to, device outage or other technical limitation, subjects may record their responses on a web back-up version of the assessments that is provided by the CRO or PRO vendor. Sites will be provided with access to the web back-up assessments as a preventative measure.

6.2.4 Allocation of Subjects to Treatment

This is an open-label, nonrandomized study where all subjects will be enrolled to receive REPLAGAL at 0.2 mg/kg EOW. Individual subject numbers are automatically assigned to all subjects via the IRT as they consent to take part in the study.

6.2.5 Dosing

REPLAGAL will be administered at a dose of 0.2 mg/kg body weight as an IV infusion over 40 minutes (±10 minutes) EOW. The first dose of REPLAGAL will be based on the subject's weight at baseline.

The appropriate dose calculation is obtained as follows: multiply the subject's weight in kg by 0.2 mg/kg. (Round up or down to the 1/10th decimal place from the 1/100th decimal place using >0.05 to round up). This will give the number of mL of drug required since the concentration is 1 mg/mL.

To determine the number of vials required, divide the number of mL determined above by 3.5 mL. Round up to the whole number and this is the number of vials required to withdraw the number of mL needed (refer to the Pharmacy Manual).

All study procedures, assessments (except for postinfusion vital signs), and sample collection should be completed prior to administering an infusion.

Ideally, REPLAGAL infusions should occur on the same day of the week, EOW (ie, every 14 days), but may occur every 14 days (±7 days) of the target day in order to facilitate subject scheduling. If at all possible, missed infusions should be avoided. If a subject is not dosed within 21 days from the scheduled dose, the subject should receive the next infusion as soon as possible. It may be acceptable to give the next infusion as early as 7 days after the previous infusion. Subsequent infusions will return to the original schedule which should be based on the subject's first infusion.

Dosing guidance will be provided in a separate study manual.

6.2.6 Dose Modification

The first dose of REPLAGAL will be based on the subject's weight at baseline. If the subject's weight changes ≥±5% from baseline weight, (or the weight last used for dose recalculation), then the subject's dose, should be recalculated based on the new weight. Weight for dose management, should be taken at the Site Visits (Weeks 12, 26, 40, 52, 64, 78, 92) as outlined in the Schedule of Activities (Table 1).

Percent weight change formula: (((W1-W0)/W0) *100)

Dose adjustments should occur as soon as possible after a weight change of ±5% is identified, but no later than the next bi-weekly infusion visit. If the dose can be adjusted prior to the infusion on the day a weight change of ±5% is identified, the dose adjustment should be made and the updated dose should be given to the subject. If the dose cannot be adjusted on the same day the weight change of ±5% is identified, the updated dose may be given at the next bi-weekly infusion.

6.3 Labeling, Packaging, Storage, and Handling of Investigational Product

6.3.1 Labeling

Investigational product labels will contain information necessary to meet the applicable regulatory requirements.

6.3.2 Packaging

Investigational product will be packaged in the following labeled containers: single-use, 5 mL vials containing 3.5 mL of concentrate at a concentration of 1 mg/mL. Changes to sponsor-supplied packaging prior to dosing may not occur without full agreement in advance by the sponsor.

6.3.3 Storage

REPLAGAL will be provided by the sponsor (or designee) to the clinical sites, qualified satellite sites, or other approved location, in a temperature-controlled, monitored container. The vials should be stored in a refrigerator at 2 to 8°C (36–46°F). A minute amount of fine particulate matter, causing the solution to appear slightly hazy, may be present during storage.

Investigational product at the clinical sites, hospitals, infusion centers, etc. must be stored in a locked refrigerator, or in a refrigerator in a locked room, with access limited to authorized study personnel.

Investigational product must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the investigational product is maintained within the established temperature range. The sponsor, or designee, must be notified of any temperature that fall out of the accepted range. Detailed guidance on storage requirements and issue reporting will be outlined in the Infusion and Pharmacy Manuals.

The sponsor should be notified immediately if there are any changes to the storage area of the investigational product that could affect the integrity of the product(s), eg, fumigation of a storage room.

6.3.4 Handling

REPLAGAL should be handled as follows:

- REPLAGAL vials should be stored in a refrigerator at 2 to 8°C (36–46°F). A minute amount of fine particulate matter, causing the solution to appear slightly hazy, may be present during storage.

- REPLAGAL is intended for IV use only.
- REPLAGAL for subject administration should be prepared by slowly mixing the appropriate amount of REPLAGAL into 100 mL of normal saline (0.9% sodium chloride) suitable for IV administration.
- Once diluted into normal saline, the solution should be rocked gently, but not shaken.
- REPLAGAL should be prepared on the day of infusion. As the product does not contain preservatives, REPLAGAL should be used as soon as possible after diluting with normal saline. Do not store above 25°C (77°F). It is recommended that infusion of the product be initiated within 24 hours of dilution.
- The diluted REPLAGAL must be administered via an IV line that contains a standard filter.
- Do not mix with or administer in conjunction with other drug solutions.
- REPLAGAL vials are intended for one time use only (3.5 mL maximum). Remaining drug product (overfill or unused drug) left in a vial after withdrawing the subject's assigned dose is **NOT** to be used for subsequent doses (refer to Pharmacy Manual).

6.4 Drug Accountability

Investigators, or designee, will be provided with sufficient amounts of the investigational product to carry out this protocol for the agreed number of subjects.

The investigator or designee will acknowledge receipt of the investigational product, documenting shipment content and condition. Accurate records of all investigational product dispensed, used, returned, and/or destroyed must be maintained for the duration of the study.

As instructed by the sponsor, at the end of the study all unused stock, and empty/used investigational product packaging may be destroyed at the site or a local facility. In this case, destruction records identifying what was destroyed, when and how, must be obtained with copies provided to the sponsor. Destruction of investigational product must be in accordance with local, state, and national laws.

In the event that a site is not allowed to destroy investigational product, all unused stock and empty/used investigational product packaging are to be sent to a nominated contractor on behalf of the sponsor. Investigational product being returned to the sponsor's designated contractors must be counted and verified by the clinical site personnel and the sponsor or their designee. The sponsor, or designee, should be contacted for authorization to return any investigational product prior to shipment. Shipment of all returned investigational product must comply with local, state, and national laws.

All investigational product should be accounted for, and the identified discrepancies should be investigated and documented to the sponsor's satisfaction. Additional guidance on drug accountability will be included in a separate study plan.

6.5 Subject Compliance

For this study, REPLAGAL is administered under controlled conditions; therefore, subject compliance with study treatment is anticipated to be high.

6.6 Prior and Concomitant Therapy

All nonstudy treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, nonpharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to screening period through the end of the follow-up period must be recorded in the subject's source documentation.

6.6.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, nonpharmacological treatment such as psychotherapy) received within 30 days prior to screening period through the date of first dose of investigational product. Prior treatment information must be recorded on the subject's source documentation.

6.6.2 Concomitant Treatment

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 treatment information must be recorded in the subject's source documentation.

6.6.3 Prohibited Treatment

The following medications and treatments are prohibited at any time prior and throughout the course of the study:

- FABRAZYME (agalsidase beta) and its biosimilars
- GLYSET (miglitol)
- ZAVESCA (miglustat)
- CERDELGA (eliglustat)
- GALAFOLD (migalastat)
- Any investigational product for treatment of Fabry disease.

The following medications should not be administered at any time during this study because these substances have the potential to inhibit intracellular GLA:

- Chloroquine
- Amiodarone
- Monobenzone
- Gentamicin

6.7 COVID-19-related Protocol Considerations

On a temporary basis, in order to maintain subject safety, confidentiality, and study integrity in the context of healthcare delivery challenges presented by the COVID-19 pandemic, subjects who may be impacted should contact study sites and investigators to determine the best course of action. Depending on the impact, in some cases it may be possible to arrange for alternative solutions as permitted by local regulations. Any decision on procedural changes should be made on a case-by-case basis by the principal investigator in consultation with the study team and the medical monitor, while maintaining subject safety and confidentiality as the priority.

Missing data, remote visits, changes to assessment approaches, and altered visit windows during the COVID-19 public health emergency may affect the study results. Thus, it is important to identify all protocol deviations and altered data collection or assessment methods. It is crucial that any deviations related to COVID-19 be clearly identified as such in the eCRF.

The following procedural changes may be considered:

- If necessary, informed consent from a potential or current trial participant may be obtained via electronic informed consent capabilities, or an electronic face-to-face consent interview when potential participants are unable to travel to the site.
- Subjects who discontinued from screening due to COVID-19-related factors may be rescreened if the medical monitor agrees
- Options for additional investigational dosing in the home setting to prevent missed doses at site visits (see Section 6.2.1)
- Remote checks instead of site visits (if appropriate) may be performed as a safety check on subject well-being
- Transfer to investigational sites away from risk zones to complete required visits

7 SCREEN FAILURE, WITHDRAWAL FROM THE STUDY, DISCONTINUATION OF STUDY TREATMENT, AND LOST TO FOLLOW-UP

7.1 Screen Failure

A screen failure is a subject who has given informed consent but who fails to meet the inclusion and/or exclusion criteria and has not been administered investigational product(s).

Subjects who screen failed or could not be enrolled during the screening period, due to circumstances including, but not limited to, COVID-19 related issues, may be rescreened with prior approval from the sponsor medical monitor. Some procedures, such as cMRI, may not need to be repeated for rescreening, with prior agreement of the sponsor medical monitor.

7.2 Withdrawal from the Study

A subject may withdraw from the study at any time and for any reason, without prejudice to his/her future medical care by the physician or at the institution, or may be withdrawn at any time at the discretion of the investigator or sponsor (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject with the medical monitor when possible. Withdrawal from the study means that the subject is no longer taking study treatment and does not return for further study visits. A subject who is withdrawn from the study does not meet the criteria to be part of the "completer set" for statistical analysis.

The investigator should make a reasonable effort to ascertain the reason(s) for withdrawal, while fully respecting the subject's right to do so. The reason for withdrawal should be recorded in the subject's source documentation. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source documentation and the most clinically relevant reason should be indicated.

Reasons for withdrawal from the study may include, but are not limited to:

- Adverse event
- Protocol deviation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Pregnancy

Subjects wishing to completely withdraw from the study should be encouraged to complete the Week 104 (end of treatment or early termination visit) and safety follow-up assessments to ensure subject safety. Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for withdrawal, date of stopping

investigational product, and the total amount of investigational product taken must be recorded in the source documents.

7.3 Discontinuation of Study Treatment

Premature Closure of the Treatment Protocol

Treatment Protocol Termination

If the sponsor or an investigator discovers conditions arising during the treatment protocol that indicate that the clinical investigation should be halted due to an unacceptable patient risk, the treatment protocol may be terminated after appropriate consultation between Shire and the investigators.

In addition, a decision on the part of Shire to suspend or discontinue development of the test material may be made at any time.

Options for Subjects upon Discontinuation from Study Treatment

In an effort to collect information on key outcomes for participants who discontinue from study treatment, all enrolled subjects who have received at least 1 dose of investigational product, and who have completed at least 1 efficacy assessment of the endpoints being analyzed, may consent to return to the clinic site for assessments conducted at the site visits. During the initial informed consent process, subjects should be reminded that, even if they choose to discontinue study treatment, their continued participation in the study is beneficial for data collection and analysis.

If for any reason investigational product is discontinued, the subject should be given the opportunity to consent to continuing study visits without being on study treatment. Subjects in this situation should review and sign a "Withdrawal of Informed Consent" document, and select from a list of options regarding their study participation. Options may include, but are not limited to:

- No longer willing/able to receive REPLAGAL study treatment, but willing to attend study visits
- No longer willing/able to receive REPLAGAL study treatment, and not willing to attend further visits, but willing to allow medical records to be consulted in future to obtain clinical information to assess the safety and efficacy of REPLAGAL
- No longer willing/able to receive REPLAGAL study treatment, and not willing to attend further visits or allow medical records to be consulted in future to obtain clinical information for REPLAGAL.

The reason for discontinuation of study treatment should be determined by the investigator and recorded in the subject's source documentation. If a subject discontinues study treatment for more than 1 reason, each reason should be documented in the source documentation and the most clinically relevant reason should be indicated. If the subject has consented to continuing in the

study, without study treatment, this outcome should also be documented in the subject's source documentation.

Reasons for discontinuation from study treatment may include, but are not limited to:

- Adverse event
- Withdrawal by subject
- Lack of efficacy
- Pregnancy
- Other (If "Other" is selected, the investigator must specify in the source document).

For subjects who discontinue study treatment but remain in the study, the End of Treatment (EOT) Visit should occur within 10-21 days after the subject's last IP dose. These subjects will also have either a Week 104 Visit (if completing the study) or an ET Visit (if withdrawing from the study prior to Week 104). Following the EOT visit, these subjects will continue with their remaining site visits as originally scheduled. Subjects should be encouraged to complete all study visits through Week 104 (end of treatment visit). The safety follow-up assessment is not necessary because the subject will already have been off study treatment, and will have had site visits at which safety has been assessed. Subjects who discontinue study treatment and complete visits through Week 104 do not meet the criteria to be part of the "completer set" for statistical analysis.

Subjects who have discontinued study treatment, and who have consented to continuing to attend study visits, can decide to withdraw from study participation at any time and for any reason, without prejudice to his/her future medical care by the physician or at the institution, or may be withdrawn at any time at the discretion of the investigator or sponsor.

Site Termination

A specific site may be terminated separate from the general treatment protocol for, but not limited to, the following conditions:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled at the site
- Insufficient adherence by the investigator to protocol or regulatory requirements

Safety Related Study Stopping Rules

The study will be considered as completed when the last participant in the study completes his/her last study assessments.

The entire study may be prematurely terminated/discontinued for the following reasons:

1. Sponsor's decision in the following cases:
    - If the information on the product leads to doubt as to the benefit/risk ratio;

- Comprehensive deficiency in the recorded data or protocol compliance so that the study cannot be reliably assessed;
- In the event of breach by the investigator of a fundamental obligation under this agreement, including but not limited to breach of the clinical study protocol, breach of the applicable laws and regulations, or breach of the ICH guidelines for GCP.

In any case, the Sponsor will notify the investigator of its decision by written notice.

2. Investigator's decision in the following case:
    - Occurrence of undesired experiences or AEs necessitating termination.

The investigator must notify the sponsor of his/her decision and give the reason in writing for premature termination/discontinuation. In all cases (decided by the sponsor or by the investigator, the IEC and Health Authorities should be informed according to applicable regulatory requirements. The principal investigator and the sponsor, if appropriate, will jointly decide premature termination with the Ethics Committee.

7.4 Lost to Follow-up

A subject is considered lost to follow-up if, after a minimum of 3 documented attempts, the site personnel are unable to make contact with the subject after their most recent study contact (site visit, infusion visit, telephone contact, etc). At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that the subject return to the clinical site for final safety assessments.

8 STUDY ASSESSMENTS AND PROCEDURES

Prior to performing any study-related procedures (including those related to screening), the investigator or his/her designee must obtain written informed consent from the subject (as per local requirements). There must be documentation of consent (as per local requirements) indicating that the subject is aware of the investigational nature of the study and the required procedures and restrictions, prior to performing any study-related procedures.

8.1 Study Periods

Refer to Table 1 for the schedule of study activities. Study assessments are detailed in Section 8.2.

8.1.1 Screening Period (Day -50 through Day -8)

The screening period starts when subjects sign informed consent. The duration of the screening period is up to 42 days where study subjects will undergo all procedures listed for the screening period in Table 1. The screening period can occur over multiple days to allow for the evaluation of subject's eligibility for inclusion in the study. Subjects who screen failed or could not be enrolled during the screening period, due to circumstances including, but not limited to, COVID-19 related issues, may be rescreened with prior approval from the sponsor medical monitor. Some procedures, such as cMRI, may not need to be repeated for rescreening, with prior approval from the sponsor medical monitor.

Screen failure is defined in Section 7.1.

8.1.1.1 Screening Visit(s)

Informed consent must be obtained before any screening procedures are performed. Screening procedures can be performed over several days. Assessments and procedures to be performed during the screening visit(s) are outlined in Table 1 and listed below.

- Obtain subject consent
- Administer pregnancy test (for women of childbearing potential only). At screening both the urine and serum β-hCG tests should be performed. The urine pregnancy test should be administered by the clinical site before any other screening procedures (except for signature of informed consent) are completed.
- Review eligibility criteria (inclusion/exclusion criteria)
- Collect samples for GLA genotyping and measurement of GLA activity for confirmation of Fabry disease (see Section 8.2.1.3)
- Collect demographic information
- Review medical history
- Perform physical examination
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature)

- Measure and collect height
- Measure weight
- Perform cMRI (will serve as the baseline value)
- Perform 12-lead ECG
- eGFR (to be calculated by a Shire-designated laboratory; if the eGFR measurement at screening is not within the range, a second eGFR measurement may be completed and, if in range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first.
- Collect blood samples to perform serum chemistry (sodium [Na+], potassium [K+], chloride [Cl-], bicarbonate [HCO3-], calcium [Ca2+], magnesium [Mg2+], phosphate, creatinine, urea nitrogen [BUN], glucose, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase [ALT], aspartate aminotransferase [AST], triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, and hematology (complete blood count: hematocrit [Hct]; hemoglobin [Hb]; RBC; WBC; platelets; basophils; basophils/total cells; eosinophils; eosinophils/total cells; immature granulocytes; lymphocytes; lymphocytes/total cells; monocytes; monocytes/total cells; neutrophils; and neutrophils/total cells) laboratory tests
- Collect urine samples for urinalysis (including proteinuria)
- Perform viral testing (including HBsAg, HBcAb, HCV antibody with confirmation by HCV-ribonucleic acid polymerase chain reaction testing, and HIV antibody)
- Record AEs
- Review of prior and concomitant medications, therapies, and medical/surgical interventions.

8.1.2 Baseline Period (Day -7 to Day 0/Week 0)

All baseline assessments and procedures are to be performed at the clinical site. The baseline assessments may be completed over a period of 7 days as long as all baseline assessments and procedures are completed prior to the first REPLAGAL infusion. The day of the first REPLAGAL infusion is considered Day 0/Week 0 and all future study visits and windows should be timed from the first infusion.

Baseline assessments and procedures are outlined in Table 1 and listed below:

- Review of inclusion/exclusion criteria
- Administer urine pregnancy test (for women of childbearing potential only). The urine pregnancy test should be administered by the clinical site before any other procedures are completed. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis.
- Measure weight
- Perform 12-lead ECG

- Collect blood samples to perform serum chemistry (sodium [Na+], potassium [K+], chloride [Cl-], bicarbonate [HCO3-], calcium [Ca2+], magnesium [Mg2+], phosphate, creatinine, urea nitrogen [BUN], glucose, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase [ALT], aspartate aminotransferase [AST], triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, and hematology (complete blood count: hematocrit [Hct]; hemoglobin [Hb]; RBC; WBC; platelets; basophils; basophils/total cells; eosinophils; eosinophils/total cells; immature granulocytes; lymphocytes; lymphocytes/total cells; monocytes; monocytes/total cells; neutrophils; and neutrophils/total cells) laboratory tests
- eGFR (to be calculated by a Shire-designated laboratory; if the eGFR measurement at screening is not within the range, a second eGFR measurement may be completed and, if in range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first.
- Collect blood samples for ADA testing
- Collect blood samples for evaluation of plasma lyso-Gb3 levels
- Collect blood samples for assessments of levels of inflammatory biomarkers
- Collect urine samples for urinalysis
- Administer the following questionnaires:
  - SF-36v2
  - BPI-SF
  - PGI-S
  - BFI
  - GSRS
- Record AEs
- Review of prior and concomitant medications, therapies, and medical/surgical interventions
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature) as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur, vital signs should still be collected. The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the subject's source documentation.
- Calculate the dose of REPLAGAL based on the subject's weight at baseline
- Administer REPLAGAL. REPLAGAL is administered EOW (±7 days) as an IV infusion over 40 minutes (±10 minutes) after all other assessments (except for postinfusion vital signs assessment), procedures and sample collection have been completed.

8.1.3 Treatment Period (Day 0/Week 0 through Week 104 or Early Termination)

The treatment period will begin after all baseline assessments and procedures are completed, and it is confirmed the subject continues to meet all inclusion/exclusion criteria. The treatment period will comprise 104 weeks during which subjects will receive every other week (EOW) IV injections of REPLAGAL. The first 3 infusions should be performed at the clinical site. (Refer to Section 6.2.1 for guidance regarding transition to home infusions.)

During the treatment period all study assessments and procedures should be completed on the day of the scheduled visit (ie, day of infusion) with the exception of visits where a cMRI will be performed (Baseline, Week 52 and Week 104 visits). On visits where a cMRI assessment will be performed, all study assessments should occur on the day of infusion; however, to accommodate the scheduling of cMRI, this assessment may be performed within ±7 days of the infusion day for Week 52 and ±14 days of the infusion day for Week 104.

Study visit windows should be calculated off of the date of the subject's first infusion of REPLAGAL.

During the Treatment Period, subjects will undergo the study procedures outlined in Table 1 and listed in the sections below.

8.1.3.1 Every Other Week Visits

The subject's first REPLAGAL infusions should be performed at the clinical site. (Refer to Section 6.2.1 for guidance regarding transition to home infusions.) Every other week visit windows should be calculated from the date of the subject's first infusion of REPLAGAL.

EOW assessments and procedures are outlined in Table 1 and listed below:

- Administer urine pregnancy test (for women of childbearing potential only). The urine pregnancy test should be administered by the clinical site before any other procedures are completed. If the urine test is positive, the infusion should not be given, and a blood sample should be collected for serum β-hCG testing and sent to the central laboratory for analysis.
- Record AEs
- Review concomitant medications, therapies, and medical/surgical interventions
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature) as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur, vital signs should still be collected. The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the source documentation.
- Administer REPLAGAL. REPLAGAL is administered EOW (±7 days) as an IV infusion over 40 minutes (±10 minutes) after all other assessments (except for postinfusion vital signs assessment), procedures and sample collection have been completed.

8.1.3.2 Week 12, Week 40, Week 64, and Week 92 Visits

Assessments and procedures at these visits are to be performed at the clinical site. These assessments and procedures are outlined in Table 1 and listed below:

- Administer urine pregnancy test (for women of childbearing potential only). The urine pregnancy test should be administered by the clinical site before any procedures are completed. If the urine test is positive, the infusion should not be given, and a blood sample should be collected and sent to the central laboratory for analysis.
- Measure weight
- Calculate REPLAGAL dose based on subject's weight
- eGFR (to be calculated by a Shire-designated laboratory)
- Collect blood samples to perform serum chemistry (sodium [Na+], potassium [K+], chloride [Cl-], bicarbonate [HCO3-], calcium [Ca2+], magnesium [Mg2+], phosphate, creatinine, urea nitrogen [BUN], glucose, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase [ALT], aspartate aminotransferase [AST], triglycerides, cholesterol, LDL cholesterol, HDL cholesterol, and hematology (complete blood count: hematocrit [Hct]; hemoglobin [Hb]; RBC; WBC; platelets; basophils; basophils/total cells; eosinophils; eosinophils/total cells; immature granulocytes; lymphocytes; lymphocytes/total cells; monocytes; monocytes/total cells; neutrophils; and neutrophils/total cells) laboratory tests
- Collect blood samples for ADA testing
- Collect blood samples for evaluation of plasma lyso-Gb3 levels
- Collect blood samples for assessments of inflammatory biomarkers
- Collect urine samples for urinalysis (including proteinuria)
- Administer the following questionnaires:
  - SF-36v2
  - BPI-SF
  - PGI-S
  - BFI
  - GSRS
- Record AEs
- Review concomitant medications, therapies, and medical/surgical interventions
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature) as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur, vital signs should still be collected.

The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the source documentation.

- Administer REPLAGAL. REPLAGAL is administered as an IV infusion over 40 minutes (±10 minutes) at the clinical site after all other assessments (except for postinfusion vital signs assessment), procedures and sample collection have been completed.

8.1.3.3 Week 26, Week 52, and Week 78 Visits

Assessments and procedures at these visits are to be performed at the clinical site. These assessments and procedures are outlined in Table 1 and listed below:

- Administer urine pregnancy test (for women of childbearing potential only). The urine pregnancy test should be administered by the clinical site before any other procedures are completed. If the urine test is positive, the infusion should not be given, and a blood sample should be collected and sent to the central laboratory for analysis.
- Perform physical examination
- Measure height at Week 52
- Measure weight
- Calculate dose of REPLAGAL based on subject's weight
- Perform cMRI for LVMI assessment (Week 52 visit, only)
- Perform 12-lead ECG
- eGFR (to be calculated by a Shire-designated laboratory)
- Collect blood samples to perform serum chemistry (Sodium [Na+], Potassium [K+], Chloride [Cl-], Bicarbonate [HCO3-], Calcium [Ca2+], Magnesium [Mg2+], Phosphate, Creatinine, Urea Nitrogen [BUN], Glucose, Albumin, Bilirubin, Alkaline Phosphatase, Alanine Aminotransferase [ALT], Aspartate Aminotransferase [AST], Triglycerides, Cholesterol, LDL Cholesterol, HDL Cholesterol, and hematology (complete blood count: Hematocrit [Hct]; Hemoglobin [Hb]; RBC; WBC; Platelets; Basophils; Basophils/Total Cells; Eosinophils; Eosinophils/Total Cells; Immature Granulocytes; Lymphocytes; Lymphocytes/Total Cells; Monocytes; Monocytes/Total Cells; Neutrophils; Neutrophils/Total Cells) laboratory tests
- Collect blood samples for ADA testing
- Collect blood samples for evaluation of plasma lyso-Gb3 levels
- Collect blood samples for assessment of inflammatory biomarkers
- Collect urine samples for urinalysis (including proteinuria)
- Administer the following questionnaires:
  - SF-36v2
  - BPI-SF

- PGI-S
- BFI
- GSRS

- Record AEs
- Review concomitant medications, therapies, and medical/surgical interventions
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature) as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur, vital signs should still be collected. The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the source documentation.
- Administer REPLAGAL. REPLAGAL is administered as an IV infusion over 40 minutes (±10 minutes) at the clinical site after all other assessments (except for postinfusion vital signs assessment), procedures and sample collection have been completed.

8.1.3.4 Week 104 Visit (End of Treatment) or Early Termination

The assessments and procedures outlined in Table 1 and listed below are to be performed at the clinical site for completers (EOT visit) or for subjects who discontinue early (early termination visit). For subjects who discontinue early, all assessments and procedures should be completed except for REPLAGAL infusion. For subjects who discontinue study treatment but remain in the study, the End of Treatment (EOT) Visit should occur within 10-21 days after the subject's last IP dose. These subjects will also have either a Week 104 Visit (if completing the study) or an ET Visit (if withdrawing from the study prior to Week 104).

- Administer urine pregnancy test (for women of childbearing potential only). The urine pregnancy test should be administered by the clinical site before any other procedures are completed. If the urine test is positive, the infusion should not be given, and a blood sample should be collected and sent to the central laboratory for analysis.
- Perform physical examination
- Subject height and weight must be measured
- Perform cMRI for LVMI assessment
- Perform 12-lead ECG
- eGFR (to be calculated by a Shire-designated laboratory)
- Collect blood samples to perform serum chemistry (Sodium [Na+], Potassium [K+], Chloride [Cl-], Bicarbonate [HCO3-], Calcium [Ca2+], Magnesium [Mg2+], Phosphate, Creatinine, Urea Nitrogen [BUN], Glucose, Albumin, Bilirubin, Alkaline Phosphatase, Alanine Aminotransferase [ALT], Aspartate Aminotransferase [AST], Triglycerides, Cholesterol, LDL Cholesterol, HDL Cholesterol, and hematology

(complete blood count: Hematocrit [Hct]; Hemoglobin [Hb]; RBC; WBC; Platelets; Basophils; Basophils/Total Cells; Eosinophils; Eosinophils/Total Cells; Immature Granulocytes; Lymphocytes; Lymphocytes/Total Cells; Monocytes; Monocytes/Total Cells; Neutrophils; Neutrophils/Total Cells) laboratory tests

- Collect blood samples for ADA testing
- Collect blood samples for evaluation of plasma lyso-Gb3 levels
- Collect blood samples for assessment of inflammatory biomarkers
- Collect urine samples for urinalysis (including proteinuria)
- Administer the following questionnaires:
  - SF-36v2
  - BPI-SF
  - PGI-S
  - BFI
  - GSRS
- Record AEs
- Review concomitant medications, therapies, and medical/surgical interventions
- Record vital signs (pulse, blood pressure, respiratory rate, and temperature) as follows: 1) within 10 minutes prior to starting the infusion; 2) 20 minutes (±5 minutes) after the start of the infusion 3) 10 minutes (±5 minutes) post infusion; and 4) 30 minutes (±5 minutes) post infusion. In the event that a scheduled infusion does not occur, vital signs should still be collected. The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the source documentation.
- Administer REPLAGAL (except for subjects who discontinue early). REPLAGAL is administered as an IV infusion over 40 minutes (±10 minutes) at the clinical site after all other assessments (except for postinfusion vital signs assessment) procedures and sample collection have been completed.

8.1.4 Follow-up Period

The safety follow-up period is 14 days (-4 days/+7 days) following EOT infusion (or the last infusion for subjects who have withdrawn from the study). At the end of this period there will be a telephone call initiated by the site staff to query for AEs, SAEs, and concomitant medications.

Subjects who experienced SAEs, or mild-worsening, moderate or severe AEs will be requested to report to the clinical site for further examination. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Appendix 3.2).

8.1.5 Additional Care of Subjects after the Study

No aftercare is planned for this study.

8.2 Study Assessments

Detailed descriptions of subject procedures and evaluations required for this protocol are described in this section. The timing for the performance of these evaluations is specified in the Schedule of Activities (Table 1).

8.2.1 Demographic and Other Baseline Characteristics

8.2.1.1 Demographics

Subject demographic information including gender, age, and race will be collected prior to the subject receiving the first dose of investigational product.

8.2.1.2 Confirmation of Study Eligibility

At screening, each subject will be reviewed for eligibility against the study inclusion/exclusion criteria. Subjects who do not meet the study entrance criteria will not be allowed to participate in the study, and the reason(s) for the subject's ineligibility will be documented; the subject will be considered a screen failure. If the eGFR measurement at screening is not within the range, a second eGFR measurement may be completed and, if in the stipulated range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first measurement.

Subject eligibility will be confirmed at baseline on the basis of review of the study entrance criteria. Subjects should not be administered study medication unless they continue to meet all inclusion/exclusion criteria at the time of dosing. For any subjects who do not continue to meet the study entrance criteria, the reason(s) for the subject's ineligibility for the study will be documented and the subject will be considered a screen failure.

8.2.1.3 Confirmation of Fabry Disease (GLA Activity and Genotyping)

Subjects will undergo evaluations at screening to confirm that they have Fabry disease. Male subjects will provide a blood sample for measurement of serum GLA activity and for genotyping of the GLA gene. Female subjects will provide a blood sample for measurement of serum GLA activity and for genotyping of the GLA gene. However, serum GLA activity is not an inclusion criterion for female subjects. Blood samples will be analyzed using a DBS assay.

8.2.1.4 Medical History

Medical history will be collected and recorded in the subject's source documents. Medical history will include a review of the subject's medical status, documentation of current and prior medical procedures, and documentation of current and prior medication usage (for details on prior medication refer to Section 6.6.1). The subject will be queried on the following:

- Relevant intercurrent illness and chronic disease update
- Disease-specific review of symptoms, including:
  - Head, neck, and thyroid
  - Eyes, ears, nose, and throat

- Chest and lungs
- Heart
- Lymph nodes
- Abdomen
- Anorectal
- Genitourinary
- Skin
- Musculoskeletal
- Endocrine
- Neurological
- Other.

8.2.2 Efficacy

8.2.2.1 Cardiac Magnetic Resonance Imaging (cMRI)

Cardiac magnetic resonance imaging will be used to assess cardiac fibrotic segments, LVMI, interventricular septal end-diastolic thickness (IVSTd), and posterior wall thickness in diastole (PWTd). Reading of images will be performed by a central reader. Image collection, preparation, and transfer instructions will be provided to the clinical sites.

8.2.2.2 Estimated Glomerular Filtration Rate (eGFR)

Estimated glomerular filtration rate will be calculated by a Shire designated laboratory from serum creatinine using the CKD-EPI equation. The CKD-EPI equation, expressed as a single equation, is:

$$eGFR = 141 * \min(Scr/\kappa,1)^{\alpha} * \max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018 \text{ [if female]} * 1.159 \text{ [if black]}$$

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.

8.2.2.3 Proteinuria

Proteinuria will be measured based on PCR. Measurement of proteinuria will be performed as part of urinalysis (see Appendix 2 for details on urinalysis).

8.2.3 Safety

8.2.3.1 Physical Examination

A full physical examination will be performed by the investigator with a thorough review of body systems on the visits specified in the Schedule of Activities (Table 1). Physical examinations will include a review of the subject's general appearance as well as evaluation of the body systems listed in Table 3. Any abnormal change in findings will be recorded as an AE on the subject's source documentation.

Table 3 Assessments for Physical Examinations

Assessment	Assessment
General appearance	Endocrine
Head and neck	Cardiovascular
Eyes	Abdomen
Ears	Genitourinary
Nose	Skin
Throat	Musculoskeletal
Chest and lungs	Neurological

8.2.3.2 Height

Subject height must be measured and collected at Screening, Week 52 and Week 104 (or early termination) visits for all subjects.

8.2.3.3 Weight

Weight is a critical measurement as it determines the dosing of REPLAGAL. Weight is initially collected at screening and again at baseline. The first dose of REPLAGAL will be based on the subject's weight at baseline. A change in subject weight of ≥±5% from baseline, or the last weight used to recalculate the dose, will require a recalculation of the dose by the clinical site. Weight will be measured every 12 weeks, as specified in the Schedule of Activities (Table 1), with any dosing adjustments completed prior to the next dose.

8.2.3.4 Adverse Events

Subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. Refer to Appendix 3 for AE definitions, assessment, collection time frame, and reporting procedures.

8.2.3.5 Vital Signs

The following vital signs will be collected at all study visits: pulse, blood pressure, respiratory rate, and temperature. On visits where a subject is scheduled to receive an infusion (baseline, EOW, and the site visits), vital signs should be recorded as outlined in (Table 4). In the event that a scheduled infusion does not occur at one of these visits, vital signs should still be collected. The vital signs that were taken within 10 minutes prior to the start of the expected infusion should be recorded in the subject's source documentation.

If there are no IRRs reported during the first 3 infusions, the observation and vital signs post infusion can be omitted at the discretion of the investigator.

The investigator will assess whether a change from baseline visit in vital signs is clinically significant, and whether the change should be considered and recorded as an AE.

Table 4 Schedule for Recording of Vital Signs at Infusion

Timing Relative to Infusion	Schedule of Assessments
Start of Infusion	Within 10 minutes prior to start of infusion
During Infusion	20 minutes (±5 minutes) after start of infusion
After Infusion[a]	10 minutes (±5 minutes) after completing the infusion 30 minutes (±5 minutes) after completing the infusion

[a] If there are no infusion-related reactions during the first 3 infusions, the observation and vital signs post infusion can be omitted at the discretion of the investigator.

8.2.3.6 Clinical Laboratory Tests (Serum Chemistry, Hematology, and Urinalysis)

All clinical laboratory tests will be performed according to the laboratory's standard procedures. Reference ranges will be supplied by the laboratory and used to assess the results for clinical significance and out-of-range changes which may be associated with, or constitute, an AE. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

A complete list of the clinical laboratory tests (serum chemistry, hematology, and urinalysis) to be performed is provided in Appendix 2.

8.2.3.7 Pregnancy Test

Female subjects of childbearing potential will have a pregnancy test administered at each study visit.

At screening, pregnancy testing will be performed using a urine test and a serum human β-hCG test. The urine pregnancy test should be administered by the clinical site before any other screening procedures (except for signature of informed consent) are completed. If the urine pregnancy test is negative, screening procedures (including the serum pregnancy test) will be completed as outlined in Table 1. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. No additional screening procedures should be completed until the result of the serum pregnancy test is available. Female subjects of childbearing potential must have a negative β-hCG serum test at screening to be eligible for the study. A positive β-hCG serum test would result in the subject being a screen failure.

At baseline, the urine pregnancy test should be administered by the clinical site before any other baseline procedures are completed. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. No additional baseline procedures will be completed until the result of the serum pregnancy test is available. If the serum β-hCG result is positive, the subject will not be dosed with REPLAGAL and would be considered a screen failure.

At all other visits during the treatment period (EOW and site visits), a urine pregnancy test will be performed prior to the completion of any study procedures. If the urine test is positive, a blood sample will be collected for serum β-hCG testing and sent to the central laboratory for analysis. The subject will not receive any additional REPLAGAL infusions until the result of the serum pregnancy test is available. If the serum β-hCG result is positive, no additional doses of the investigational product are to be administered. Pregnancy should be reported to Shire Global Patient Safety Evaluation, and the Shire medical monitor (or designee) within 24 hours of becoming aware of the pregnancy. For more details on pregnancy reporting refer to Appendix 3.8.

8.2.3.8 Electrocardiogram

Twelve-lead ECGs will be performed in accordance with the clinical site's standard practice(s). ECG recordings will be read locally at the clinical site by the investigator or a qualified designee. ECGs will include assessment of heart rate, sinus rhythm, atrial or ventricular hypertrophy, and assessment of PR, QRS, QT, and corrected QT intervals (Bazetts and Fridericia). Identification of any clinically significant findings and/or conduction abnormalities will be recorded in the source documentation.

8.2.3.9 Anti-drug Antibody Testing

Blood samples will be collected for all subjects for the determination of anti-agalsidase alfa antibodies prior to the subject receiving the REPLAGAL infusion at time points specified in the Schedule of Activities (Table 1). Blood samples collected for anti-agalsidase alfa antibody determination will be evaluated at a research laboratory designated by the Sponsor. These samples will be analyzed for anti-agalsidase alfa binding antibodies. Confirmed antibody-positive samples will undergo assessment for NAbs. Sample collection, processing, and shipping instructions will be detailed in the study laboratory manual.

8.2.3.10 Prior/Concomitant Medications, Therapies, and Medical/Surgical Interventions Assessments

Prior medications received within 30 days prior to screening through the date of first dose of investigational will be reviewed and recorded in the subject's source documentation (refer to Section 6.6.1 for full details on prior treatments).

All medications, therapies/interventions administered to and medical/surgical procedures performed on the study subjects from the time of first dose investigational product through the end of the safety follow-up period are regarded as concomitant and will be recorded on the subject's source documentation (Refer to Section 6.6.2 for full details on collection of concomitant treatments).

8.2.3.11 Viral Testing

Viral testing will be performed at screening to ensure that subjects do not present with viral infections that might compromise their ability to safely complete the study and confound later interpretation of the study findings. Viral tests include HBsAg, HBcAb, HCV antibody with confirmation by HCV-ribonucleic acid polymerase chain reaction testing, and HIV antibody.

8.2.4 Other

8.2.4.1 Investigational Product Administration

Prior to the first infusion of REPLAGAL, all baseline assessments and procedures must be completed. REPLAGAL's administration will occur as described in Section 6.2 at the visits specified in Schedule of Activities (Table 1) after all other assessments (except for post-infusion vital signs) procedures and sample collection have been completed.

The first 3 REPLAGAL infusions (including baseline infusion) as well as infusions at Week 12, Week 26, Week 40, Week 52, Week 64, Week 78, Week 92, and Week 104 visits are to be administered at the clinical site. After the first 3 doses, subjects who have not experienced a treatment-related SAE or a moderate or severe IRR may receive their subsequent infusions at home by qualified and trained medical personnel, per the discretion and direction of the investigator. If at any point during home infusions subjects experience a treatment-related SAE or a moderate or severe IRR, their next infusion should be administered at the clinical site. Infusions should continue at the clinical site until no further moderate or severe IRRs are reported, at which point subjects may be re-evaluated for consideration to retransition to home infusions (for more details on home infusions refer to Section 6.2.1).

8.2.4.2 Pharmacodynamics

Globotriaosylsphingosine (Lyso-Gb3)

Pharmacodynamic assessment will be performed by measuring lyso-Gb3 levels in plasma. Lyso-Gb3 plasma samples will be analyzed at a Shire-designated laboratory using a validated assay.

Inflammatory Biomarkers

In order to assess the impact of REPLAGAL on serum levels of inflammatory biomarkers relevant to Fabry disease, samples will be collected and tested including but not limited to INTLK1β, TNF-α, VCAM1, and ICAM1.

8.2.4.3 Health-related QoL Assessments

The study will include the following a health-related QoL assessment which will be administered at the baseline, Week 12, Week 26, Week 40, Week 52, Week 64, Week 78, Week 92, and Week 104 visits. In the case of an unplanned event that disallows/prevents the administration of the PRO on a device, for reasons including but not limited to, device outage or other technical limitation, subjects may record their responses on a web back-up version of the assessments that is provided by the CRO or PRO vendor. Sites will be provided with access to the web back-up assessments as a preventative measure.

Further information concerning these assessments is provided in Appendix 5.

Short Form-36 Health Survey (SF-36v2)

The SF-36v2 is a multipurpose, valid, short-form health survey comprising 36 questions. It consists of eight scaled scores, which are the weighted sums of the questions in their section.

Each scale is directly transformed into a 0 to 100 scale on the assumption that each question carries equal weight.

The higher the score the less disability ie, a score of zero is equivalent to maximum disability and a score of 100 is equivalent to no disability. The 8 domains assessed are: physical functioning, role limitations due to physical problems functioning, bodily pain, general health perceptions, vitality, physical role functioning, social role functioning, role-limitations due to emotional problems role functioning, and mental health. The questionnaire is expected to take 5 minutes to be completed (Ware and Sherbourne 1992).

Brief Pain Inventory-Short Form (BPI-SF)

The BPI-SF is a 9-item self-administered questionnaire that will be used to evaluate the severity of a subject's pain and the impact of this pain on the subject's daily functioning. The subject will be asked to rate their worst, least, average, and current pain intensity, list current treatments and their perceived efficacy, and rate the degree that pain interferes with general activity, mood, walking ability, normal work, relations with other persons, sleep, and enjoyment of life on a 10-point scale. The questionnaire is expected to take about 5 minutes to be completed (Keller et al. 2004).

Patient Global Impression of Fabry Symptom Severity (PGI-S)

The PGI-S is a validated 1-item questionnaire designed to assess subjects' impression of Fabry symptom severity. The subject has to select the one response from the response options that gives the most accurate description of his/her state of health (overall status). The questionnaire is expected to take about 5 minutes to be completed (Farrar et al. 2001; Guy 1976).

Brief Fatigue Inventory (BFI)

The BFI is a 9-item questionnaire developed to assess the severity of fatigue and its impact on daily functioning. Each question asks the respondent to rate the level of their experienced fatigue on an 11-point (0–10) scale. The first question measures fatigue severity at current level while the following two questions rate usual and worst fatigue levels over the past 24 hours. In these 3 questions 0 indicates "no fatigue" and 10 indicates fatigue "as bad as you can imagine". The following six questions assess the level of fatigue interference with daily activities including general activity, mood, walking ability, normal work (both inside and outside the home), relations with other people, and enjoyment of life. A score of 0 corresponds to no interference while a score of 10 indicates complete interference. The questionnaire is expected to take 5 minutes to be completed (Mendoza et al. 1999).

Gastrointestinal Symptom Rating Scale (GSRS)

The GSRS is a disease-specific instrument based on a previous week recall period. It comprises 15 items that combine into five symptom clusters: reflux, abdominal pain, indigestion, diarrhea, and constipation. Each item is rated on a 7-point Likert scale. The reliability and validity of the GSRS are well-documented across a wide variety of GI conditions, and norm values for a general population are available. The questionnaire is expected to take about 5 minutes to be completed (Dimenäs et al. 1993; Dimenäs et al. 1995; Kulich et al. 2008; Svedlund et al. 1988).

8.2.5 Retention of Testing Samples

Antibody and biomarker analyses may be performed at a research laboratory designated by the Sponsor. Collection and processing of samples will be performed as specified in the Laboratory and/or Study Operations Manuals. Samples will be stored securely to ensure subject confidentiality. Samples obtained for this study may be stored for up to 10 years after the end of the study and may be used for further analyses. Thereafter, samples will be destroyed.

8.2.6 Volume of Blood to Be Drawn From Each Subject

Table 5 Volume of Blood to Be Drawn From Each Subject

Assessment		Sample Volume (mL)	Number of Samples	Total Volume (mL)
Safety	Biochemistry	6	10	60
	Hematology	2	10	20
	β-hCG[a]	2	1	2
	ADA[b]	4	9	36
	Viral testing (HBsAg, HIV, HCV)	6	1	6
Confirmation of Fabry disease (GLA activity and genotyping)		1	1	1
Inflammatory biomarkers (INTLK1β, TNF-α, VCAM1, ICAM1)		5	7	35
Lyso-Gb3		3	7	21
Total (mL)		29		181

ADA=antidrug antibody; β-hCG=beta-human chorionic gonadotropin; GLA=α-galactosidase A; HBsAg=hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus; ICAM1=Intercellular Adhesion Molecule 1; INTLK1β=Interleukin 1 Beta; lyso-Gb3=globotriaosylsphingosine; TNF-α=Tumor Necrosis Factor; VCAM1=Vascular Cell Adhesion Molecule 1

[a] β-hCG testing for females only.

[b] Any samples that are confirmed positive for ADA will also be tested for neutralizing antibodies (NAbs).

During this study, it is expected that approximately 181 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 181 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, investigational product exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused, and spurious data will be addressed.

The first version of the SAP will be finalized before the enrollment of the first patient. To preserve the integrity of the statistical analysis and study conclusions, the SAP will be finalized prior to database lock.

All statistical analyses will be performed using SAS® software (SAS Institute, Cary, NC 27513) version 9.3 or higher.

9.2 Planned Interim Analysis, Adaptive Design, and Data Monitoring Committee

No interim analysis, adaptive design, or data monitoring committee is planned for this study.

9.3 Sample Size and Power Considerations

The primary endpoints for this study are change in eGFR and LVMI from baseline up to Week 104. Two publications by Schiffmann et al. (Schiffmann et al. 2009) and Kampmann et al. (Kampmann et al. 2008) are acknowledged as landmark references reporting on the natural history of renal and cardiac function disease progression in untreated subjects with Fabry disease, respectively. The results of these studies are used across the Fabry disease medical community as a reference for the assessment of therapeutic expectations and the response of treatment. Use of these published, aggregate data as reference values for untreated changes in the endpoints have some limitations, as the adjustment for bias in patient baseline characteristics is not feasible without access to individual patient data. Nonetheless, this relevant publicly available data will provide necessary information to evaluate the REPLAGAL efficacy benefit.

Beck et al. (Beck et al. 2015) describes the disease progression in cardiac and renal function for untreated subjects with Fabry disease based on the data reported by Schiffmann et al. (Schiffmann et al. 2009) and Kampmann et al. (Kampmann et al. 2008), respectively. In this untreated population, the mean weighted annualized rate of change for eGFR and LVMI is approximately -3 mL/min/1.73 m^2 and +5 $g/m^{2.7}$, respectively, which is equivalent to a change of -6 mL/min/1.73 m^2 and +10 $g/m^{2.7}$, respectively over a period of 104 weeks. Based on the clinical document of Summary of Clinical Efficacy provided by Shire in the REPLAGAL New Drug Submission for Health Canada, the standard deviation for change in eGFR and LVMI in ERT-naïve subjects following 24-month treatment with REPLAGAL is 7 mL/min/1.73 m^2 and 15 $g/m^{2.7}$, respectively.

The null hypothesis for eGFR is that the mean change from baseline in eGFR at Week 104 of the treated subjects is less than or equal to -6 mL/min/1.73 m^2, the mean change of untreated subjects. The alternative hypothesis states that the mean change in eGFR is greater than this reference value of -6 mL/min/1.73 m^2. The null hypothesis for LVMI is that the mean change from baseline in LVMI at Week 104 of the treated subjects is greater than or equal to +10 $g/m^{2.7}$, the mean change of untreated subjects. The alternative hypothesis states that the mean change in LVMI is smaller than this reference value of 10 $g/m^{2.7}$.

The Bonferroni adjustment to control the type I error rate for the 2 primary endpoints is incorporated in the sample size determination. The Bonferroni adjustment does not require any assumptions of the two primary endpoints. If both endpoints are significant at the 0.025 significance level, then the power will be large than quoted below.

For eGFR, with 36 evaluable subjects, the study has at least 90% power to detect a decrease after 104 weeks of treatment of 1.7 mL/min/1.73 m^2 compared with a reference untreated change of -6 mL/min/1.73 m^2 with SD=7 (effect size of 0.61) using a 1-sided one sample t-test at significance level of 0.0125. For LVMI, with 36 evaluable subjects, the study has at least 90% power to detect an increase after 104 weeks of treatment of 0.64 $g/m^{2.7}$ compared with a reference untreated change of 10 $g/m^{2.7}$ with SD=15 (effect size of 0.62) using a 1-sided one sample t-test at significance level of 0.0125.

It is anticipated that 45 subjects will need to be enrolled in the study in order to obtain 36 evaluable subjects, ie, subjects who have not discontinued from investigational product and who have valid, nonmissing values of primary efficacy endpoints (eGFR and LVMI) for baseline and Week 104 assessments. The anticipated percentage of subjects enrolled who will not meet the criteria to be considered as part of the completers set (approximately 20%) is based on data from previous Shire-sponsored studies with REPLAGAL, studies with competitor drugs in the Fabry disease area, and other figures reported in published literature.

In a recently completed Shire-sponsored study (HGT-REP-081) with REPLAGAL, 27 of 167 enrolled subjects discontinued from the study, representing a drop-out rate of 16%. Two other recently published studies, ATTRACT and FACETS, provide recent discontinuation data in patients with Fabry disease. In ATTRACT (Hughes et al. 2017), a 9% drop-out rate was observed at 18 months, but a much higher rate of 16% was reported in patients who entered the 12-month open-label extension. In FACETS (Germain et al. 2016), a 20% drop-out rate was noted among migalastat-randomized patients after 24 months of treatment. Although not specific to Fabry disease, a review (Wood et al. 2004) of 71 randomized clinical studies published in leading medical journals found that 18% of the studies had a drop-out rate of 20% or higher (ie, studies published in specialist journals show that the drop-out rate is likely to be higher).

It is anticipated that the majority of subjects who do not meet the criteria to be considered as part of the completers set will have data that can be meaningful for analysis. Enrolled subjects who have received at least 1 dose of investigational product and who have completed at least 1 efficacy assessment of the endpoints being analyzed will be part of the modified intent-to-treat (mITT) set (see Section 9.4). The mITT set will be used for all analyses related to primary efficacy, secondary efficacy, inflammatory biomarker, and PRO endpoints.

The study population will target approximately 40% females and 40% males for enrollment.

9.4 Statistical Analysis Sets

The statistical analysis will include the following analysis sets:

- The **intent-to-treat (ITT)** set (those subjects who are considered enrolled in the study) will include all subjects who sign informed consent form and are eligible for the study based on the defined inclusion exclusion criteria. There is a possibility that a subject is enrolled in the study but does not receive a dose of investigational product.
- 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 efficacy assessment of the endpoints being analyzed.
- The **safety set** will include all subjects in the ITT set who receive at least 1 dose of investigational product. The safety set will be used for the analysis of safety endpoints.
- The **per-protocol (PP) set** will include all subjects in the ITT 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 Shire.
- The **completers set** will include all subjects in the ITT who have valid, values of primary efficacy endpoints (eGFR and LVMI) for both baseline and Week 104 assessments.

9.5 Efficacy Analyses

9.5.1 Primary Efficacy Endpoint

The primary efficacy endpoints are:

- Change from baseline at Week 104 in renal function, assessed by eGFR (using the CKD-EPI formula)
- Change from baseline at Week 104 in cardiac structure, assessed by LVMI using cMRI.

The primary analysis for change from baseline at Week 104 in eGFR will be based on a mixed-effects model for repeated measures (MMRM) with multiple imputation under the assumption missing is not at random using the ITT population. A Bonferroni adjustment will be used to control the overall one-sided type I error rate of 0.025 for 2 primary endpoints. The primary null hypothesis to be tested for eGFR is that the mean change from baseline in eGFR at Week 104 of treated subjects is less than or equal to -6 mL/min/1.73 m^2 , the mean change of reference untreated subjects. The alternative hypothesis is that the mean change in eGFR is greater than -6 mL/min/1.73 m^2 , the mean change of the reference untreated subjects. A one-sided p-value to test if the mean change is greater than -6 mL/min/1.73 m^2 from MMRM model will be used to interpret that there is sufficient evidence to reject the null hypothesis. The model will include visit as the fixed effect; with adjustment for sex (male versus female), age at

baseline and baseline eGFR. In the model, visit will be treated as a class variable, assuming an unstructured covariance matrix to model the within subject variability.

The primary analysis for change from baseline at Week 104 in LVMI will be based on a MMRM with multiple imputation under the assumption that missing is not at random (MNAR) using the ITT population. The primary null hypothesis to be tested for LVMI is that the mean change from baseline in LVMI at Week 104 of treated subjects is greater than or equal to 10 $g/m^{2.7}$, the mean change of the reference untreated subjects. The alternative hypothesis is that the mean change in LVMI is less than 10 $g/m^{2.7}$, the reference value of untreated subjects. A one-sided p-value to test if the mean change is less than 10 $g/m^{2.7}$ from MMRM model will be used to interpret that there is sufficient evidence to reject the null hypothesis. The model will include visit as the fixed effect; with adjustment for sex (male versus female), age at baseline and baseline LVMI. In the model, visit will be treated as a class variable, assuming an unstructured covariance matrix to model the within subject variability.

Missing eGFR and LVMI data due to intercurrent event or other reasons will be handled according to the treatment policy strategy. Treatment policy strategy is defined as the strategy that considers "The occurrence of the intercurrent event is irrelevant: the value for the variable of interest is used regardless of whether or not the intercurrent event occurs." (per ICH E9 R1 Addendum). Intercurrent events can include but are not limited to the following: (1) discontinuation of study drug, (2) discontinuation of the study, or (3) initiation of a new treatment. In a situation that missingness of the eGFR or LVMI data cannot be predicted solely based on subject's observed data, such missingness will not be considered as missing at random, but rather as MNAR. The missing pattern will be evaluated and subjects with and without missing data will be compared to gain understanding of the nature of the missingness. Pattern-mixture models and Monte Carlo Markov Chain methodology will be implemented in multiple imputation with a list of observed variables and covariates to impute missing eGFR and LVMI data with the assumption of MNAR. The covariates can be variables related to missingness identified in the comparison of subjects with and without missing data, the covariate can also be variables related to eGFR or LVMI based on medical input.

The analyses of the primary endpoints will be performed with all available data through Week 104. The repeated measure analyses will be based on the restricted maximum likelihood method assuming an unstructured covariance matrix to model the within-subject errors. If the analysis fails to converge, a compound symmetry covariance structure will be used to model the within-subject errors. Denominator degrees of freedom for the F test for fixed effects will be estimated using the Kenward-Roger approximation.

The following analyses will be performed for the primary endpoints to evaluate the robustness of the results from the primary analysis methods:

- MMRM analyses using the completers set
- MMRM using the PP set
- The eGFR and LVMI annualized rate of change and 95% CI will be estimated for subjects using the random intercept and slope model. Baseline eGFR or baseline

LVMI, age at baseline, sex, and treatment duration on investigational product in year will be included in the model.

- Tipping point analysis with multiple imputation approach will be conducted for analyses of change from baseline in eGFR or LVMI at week 104 in ITT.

Descriptive summary statistics including the number of subjects (n) and mean (standard deviation [SD]) of raw values of each the primary endpoints and change from baseline at each visit will be presented by visit. The analysis of efficacy endpoints should be based primarily on the ITT and mITT sets, and subsequently on completers and PP sets.

Descriptive summary statistics for each of the primary endpoints will also be provided by sex subgroups.

9.5.2 Secondary Efficacy Endpoints

The same model used for the primary endpoints will be used. The secondary efficacy endpoints are:

- Annualized rate of change in eGFR
- Annualized rate of change in LVMI
- Change over time in eGFR
- Change over time in LVMI
- Change over time in proteinuria, measured by PCR
- Change over time of the number of fibrotic segments suggestive of cardiac fibrosis as assessed by volume of fibrosis, measured by cMRI
- Change over time in IVSTd and PWTd, measured by cMRI
- Change over time in plasma lyso-Gb3.

For other secondary efficacy endpoints, summary statistics of the raw values and change from baseline will be provided descriptively by visit. Simple summary statistics will be produced initially. Continuous variables will be summarized using the following descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. The number and percentage of observed levels will be reported for all categorical measures. Subsequently, MMRM will be performed to explore the changes of the secondary efficacy endpoints over the course of follow-up. MMRM will be calculated using unstructured covariance (subject level) and restricted maximum likelihood estimation (REML). The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each secondary efficacy endpoint to account for any prespecified possible baseline confounder.

All analyses related to secondary efficacy endpoints will be performed using the ITT and mITT sets.

9.5.3 Multiplicity Adjustment

The following strategies will be employed to alleviate the issue of multiple testing in this study:

- Defining several primary endpoints creates a multiplicity problem. Testing each endpoint separately at $\alpha=0.05$ would inflate the Type I error and overstate the statistical significance. Therefore, failure to account for multiplicity can lead to false inference regarding the effects of a drug. To address the multiplicity problem, a Bonferroni adjustment will be implemented.
- Apart from the two primary endpoints, all other endpoints will be considered to be secondary, and any significant finding amongst these outcomes will not therefore be interpreted as confirmatory.
- Prespecification of analysis populations, subgroups of interest, methods for calculating and analyzing each outcome, would reduce the potential for multiplicity. To this end, all these issues will be described and documented in the SAP prior to any statistical analysis.

9.5.4 Control of Type I Error

A Bonferroni adjustment will be used to control the overall one-sided type I error rate.

9.5.5 Exploratory Efficacy Endpoints

The exploratory efficacy endpoint is the change over time in serum levels of inflammatory biomarkers including but not limited to INTLK1β, TNF-α, VCAM1, and ICAM1.

Concentrations of inflammatory biomarkers will be summarized using descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. Initially, simple summary statistics will be produced for each inflammatory biomarker. Subsequently, MMRM will be performed to explore the changes of INTLK1β, TNF-α, VCAM1, and ICAM1 over the course of follow-up. MMRM will be calculated using unstructured covariance (subject level) and REML. The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each inflammatory biomarker to account for any prespecified possible baseline confounder.

All analyses related to inflammatory biomarkers will be performed using the ITT and mITT sets.

9.6 Safety Analyses

The safety endpoints include:

- AEs
- ADA assessments, including NAbs for samples confirmed positive for ADA
- Clinical laboratory tests (serum chemistry, hematology, and urinalysis)
- Electrocardiography
- Vital signs.

All safety analyses will be presented for the safety set. All safety measures including, vital signs, clinical laboratory results (hematology, chemistry, urinalysis), ECG, and ADA (including NAbs) assessments will be summarized descriptively at baseline and for each postbaseline visit. Shift from baseline will be provided, if applicable.

Treatment emergent adverse events (TEAEs) are defined as AEs that start or deteriorate during or after the first dose of investigational product through the safety follow up visit/contact. The number of events, incidence, and percentage of TEAEs will be calculated overall and by system organ class and preferred term at both subject level and adverse event level. TEAEs will be further summarized by severity and relationship to investigational product. 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 number of subjects commencing follow-up in the relevant analysis population, irrespective of dropouts during the course of follow-up. Adverse events related to investigational product including IRRs, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

9.7 Other Analyses

9.7.1 Health-related Quality of Life Analyses

The change over time in subject rating of pain, fatigue, severity of global Fabry symptoms and quality of life will be analyzed as exploratory endpoints using the following PRO measures, respectively:

- SF-36v2
- BPI-SF
- PGI-S
- BFI
- GSRS

Initially, simple summary statistics will be produced for the overall score and (if available) any subscale score of each PRO measure. Continuous variables will be summarized using the following descriptive statistics: mean, SD, median, first and third quartiles, minimum and maximum. The number and percentage of observed levels will be reported for all categorical measures. Subsequently, MMRM will be performed to explore the changes of PRO measures over the course of follow-up. MMRM will be calculated using unstructured covariance (subject level) and REML. The MMRM will include the fixed effect of visit. Significance tests will be based on least-squares means. Further on, if considered necessary, the MMRM will be repeated for each PRO measure to account for any prespecified possible baseline confounder.

All analyses related to PRO measures will be performed using the ITT and mITT set.

9.7.2 Sensitivity Analysis

The assumption of normality in continuous endpoints will be assessed, and, in case of deviations, a sensitivity analysis will be conducted using log-transformed values. Regarding outliers, the primary endpoints are expected to be within the following ranges: (a) eGFR: 5-200 mL/min/1.73m², (b) LVMI: 10-150 g/$m^{2.7}$. Depending on the extent of outliers, a sensitivity analysis might be considered necessary. Subgroup analyses aiming to assess whether the observed effect is consistent across underlying patient subgroups can be viewed as some form of sensitivity analysis (ie, robustness across subgroups). Such analyses are planned to be conducted according to key baseline characteristics (eg, age, gender). These subgroup analyses will be performed through inclusion of an interaction term (ie, of the subgroup variable x main exposure variable) in the regression models. If, after performing sensitivity analyses, the findings are consistent with those from the primary analysis and lead to reasonably similar estimates, this provides assurance on the robustness of the results and lead to the conclusion that the underlying factors had little or no influence or impact on the primary estimates.

9.8 Missing, Unused, and Spurious Data

Some degree of missing data is expected for both baseline characteristics and outcomes for this study. Subjects may withdraw from the study prior to completion of the intended follow-up period for reasons such as death, severe intercurrent illness, withdrawal of consent, and loss to follow-up. For each case of premature termination, detailed information will be obtained and documented in the final report explaining circumstances leading to the termination. Nevertheless, with a sample size of 45 subjects, losing a significant percentage of the data can have drastic effects on statistical power and vastly increase Type-II error rates.

For each endpoint, a decision will be made as to whether special methods of analysis need to be employed to handle missing data on an endpoint-by-endpoint basis, and this will be documented in the SAP before the final analysis. The decision will be based upon the extent of missing data, the likelihood of bias occurring as a result of missing data, and the importance of each endpoint. Specific rules will be applied to impute missing dates depending on whether whole date (year, month, and day) is missing or date is partially missing (month or day); start date or ad-hoc date is missing. All different scenarios of missing dates imputation will be presented in detail in the SAP.

Providing that the missing data are not extensive, and that data are not missing selectively, the mixed-effects model for repeated measures (MMRM) will be employed to enable all continuous endpoints or scores assessed at several time points to be included in the analysis, rather than only subjects with nonmissing values for all time points. In the presence of missing repeated measures for a subject, the MMRM analysis does not exclude this subject from the analysis; instead, it uses all the available data. If missing data are substantial, the data will be analyzed, in addition to the prespecified methods, with multiple imputation (MI), a Monte Carlo technique in which the missing values are replaced by m>1 simulated versions, where m is typically small (eg, 3-10). The question of how to obtain valid inferences from imputed data will be addressed according to Rubin's method for `repeated imputation' inference. The advantage of the method is that once the imputed data set has been generated, the analysis can be carried out using standard statistical procedures in virtually any statistical package.

If results obtained from models using different missing mechanisms (eg, multiple imputation versus MMRM) are not similar, full details of the differences between these models should be provided in the final report. In any case the impact different settings of a model have on the results obtained will be explained in detail. The sponsor acknowledges that both procedures (MMRM and MI) assume that the missing data are missing at random (MAR), an assumption that should be generally checked for plausibility before its use. Nevertheless, it would be difficult to test or to verify a systematic pattern for a MNAR mechanism in the context of the current study. To address this issue, and in case the amount of missing data is substantial, subjects with missing versus subjects with nonmissing primary endpoints will be compared in terms of demographic and baseline characteristics such as gender, age, and relevant clinical factors, and (if any) noteworthy differences will be documented and investigated.

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11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

Appendix 1 Regulatory, Ethical, and Study Oversight Considerations

Appendix 2 Clinical Laboratory Tests

Appendix 3 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow Up, and Reporting

Appendix 4 Contraceptive Guidance

Appendix 5 Scales and Assessments

Appendix 6 Abbreviations

Appendix 7 Protocol History

Appendix 1 Regulatory, Ethical, and Study Oversight Considerations

Appendix 1.1 Regulatory and Ethical Considerations

This study is conducted in accordance with current applicable regulations including ICH E6, as well as other country and local ethical and legal requirements, and all updates.

Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the Declaration of Helsinki.

The name and address of each third-party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

Appendix 1.2 Sponsor's Responsibilities

Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, current ICH GCP Guidelines, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of investigational product for shipment to the site.

Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the applicable parties as necessary.

Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

The timing for study registration and results summary posting must be in accordance with applicable local and national requirements.

Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 24 weeks of study completion date for pediatric studies and within 1 year for nonpediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies, and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.

Appendix 1.3 Investigator's Responsibilities

Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996) and E6 R2 (2017), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and subinvestigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

Protocol Adherence and Investigator Agreement

The investigator and any subinvestigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all investigational product, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

Documentation and Retention of Records

Case Report Forms

Electronic Case Report Forms will be completed for each subject and should be handled in accordance with instructions from the sponsor or designee.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded into eCRFs. The eCRFs have been designed to record all observations and other data pertinent to the clinical investigation. The clinical research associate (CRA)/study monitor will verify the contents of the eCRFs against the source data per the monitoring plan. If data is unclear or contradictory, queries are sent for corrections or verification of data.

Case report forms must be completed by the investigator or designee as stated in the site delegation log. The investigator is required to sign the eCRF after all data have been captured for each subject.

Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to, subject's medical file, original clinical laboratory reports, and imaging reports.

All key data must be recorded in the subject's source documents.

The investigator must permit authorized representatives of the sponsor; the respective national, local, or foreign regulatory authorities; the IRB/EC; and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC, or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, X-rays etc).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US Food and Drug Administration [FDA], European Medicines Agency [EMA], United Kingdom [UK] Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

Appendix 1.4 Data Management Considerations

Data Collection

This study will be monitored according to GCP.

The investigators' authorized site personnel must enter the information required by the study eCRF Completion Guidelines or similar for all data requiring transcription of the source. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. The study will be monitored according to Good Clinical Practice.

At each visit during the baseline and treatment periods, patient-facing assessments including the SF-36, BPI-SF, PGI-S, BFI, and GSRS will be collected via an electronic device. These assessments will be captured on the device and transmitted to a third-party database where site staff will have access to the patient data via a restricted website.

Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan or similar. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

Data Handling

No special data handling will be performed for this study.

Appendix 1.5 Ethical Considerations

Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form that was reviewed by the IRB/EC and received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/ Ethics Committee (EC) for review, and all must be approved prior to site initiation.

The applicant for an EC opinion can be the sponsor or investigator for sites within the EU; for multicenter studies, the applicant can be the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement. Investigational product supplies will not be released until the sponsor, or designee has received written IRB/EC approval.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue. If required by local law, substantial amendments to the protocol must also be approved by the appropriate regulatory agency prior to implementation.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol at least annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. This can be the responsibility of the sponsor or investigator for sites within the EU; or for multicenter studies, the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs as required by IRB/EC procedures.

Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the Health Insurance Portability and Accountability Act (HIPAA) of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the sponsor or designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market Replagal; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities. Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected, if permitted under local laws governing privacy.

The results of studies containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth, where allowed per local law, may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

Study Results/Publication Policy

The term "Publication" shall mean any paper, article, manuscript, report, poster, internet posting, presentation slides, abstract, outline, video, instructional material, presentation (in the form of a written summary), or other public disclosure of the study results, in printed, electronic, oral, or other form. The parties understand and agree that participation in the study may involve a commitment to publish the data from all sites participating in the study in a cooperative publication with other investigators prior to publication or oral presentations of the study results on an individual basis. The site agrees not to publish or present the site's study results until such time as either the aggregate multisite study results are published in a cooperative publication or for a period of one (1) year after termination or completion of the study at all participating sites, whichever shall first occur. After that time, the site may publish the site's study results in scientific journals or present the study results at symposia or other professional meetings in accordance with the following provisions:

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single site data being presented.

At least sixty (60) days prior to submitting an abstract, manuscript, or other document for publication, a copy of the proposed publication will be provided to the sponsor by the site for review. Upon the sponsor's request, the site agrees to remove any and all confidential information (expressly excluding study results) identified in the publication and to delay such submission or presentation for an additional sixty (60) day period in order to allow the sponsor time to file any patent application(s). All publications of the study results shall appropriately reference the multisite study publication, if any, or the fact that the study results are a subset of data resulting from a larger multisite study.

Shire is committed to transparent dissemination of all scientific, technical, and medical manuscripts generated from Shire-supported research. Therefore, after January 1, 2018, Shire will require the submission of all Shire-supported research manuscripts to journals that offer public availability via Open Access (including publisher platforms/repositories and self-archiving). Open Access refers to the free at point of entry, online availability of published research output with, where available, rights of reuse according to an End User License.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) Recommendation for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical journals. Participation as an investigator does not confer any rights to authorship of publications.

Appendix 2 Clinical Laboratory Tests

The following clinical laboratory assessments will be performed:

Serum Chemistry

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Calcium
- Magnesium
- Phosphate
- Creatinine
- Urea Nitrogen
- Glucose
- Albumin
- Bilirubin
- Alkaline phosphatase
- Alanine Aminotransferase
- Aspartate Aminotransferase
- Triglycerides
- Cholesterol
- LDL Cholesterol
- HDL Cholesterol

Hematology

- Hemoglobin
- Hematocrit
- Platelets
- RBC
- White blood cell count with differential

Urinalysis

- pH
- Creatinine
- Albumin
- Protein

Appendix 3 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Appendix 3.1 Adverse Event Definitions

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this investigational product or medicinal product. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not causality is suspected (ICH Guidance E2A 1995).

Treatment-emergent Adverse Event

A 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.

Serious Adverse Event

A SAE is any untoward clinical manifestation of signs, symptoms or outcomes whether considered related to investigational product or not and at any dose:

- Results in death
- Is life-threatening. Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of hospitalization. Note: Hospitalizations that are the result of elective or previously scheduled investigations procedures or surgery for pre-existing conditions and have not worsened after initiation of treatment should not be classified as SAEs.

  For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Results in a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include bronchospasm associated with anaphylaxis requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; the development of drug dependency or drug abuse; and reviewed and confirmed seroconversion for human immunodeficiency virus (HIV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis E virus (HEV), or parvovirus B19 (B19V).

Unexpected Adverse Event

An unexpected adverse event is an AE whose nature, severity, specificity, or outcome is not consistent with the term, representation, or description used in the Reference Safety Information (RSI). "Unexpected" also refers to the AEs that are mentioned in the investigator's brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the product, but are not specifically mentioned as occurring with the particular product under investigation.

The expectedness of AEs will be determined by the sponsor using the investigator's brochure as the RSI. This determination will include considerations such as the number of AEs previously observed, but not on the basis of what might be anticipated form the pharmacological properties of a product.

Suspected Unexpected Serious Adverse Reaction

A Suspected Unexpected Serious Adverse Reaction is defined as any suspected adverse reaction to study treatment (ie, including active comparators) that is both serious and unexpected.

The event(s) must meet all of the following:

- Suspected adverse reaction
- Serious
- Unexpected
- Assessed as related to study treatment.

Infusion-Related Reactions

Definition of Infusion-Related Reactions

Infusion-related reactions 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 treatment. Adverse events that are considered IRRs will be noted as such in the subject's source documentation. Other AEs that occur prior to an infusion, along with AEs associated with protocol-defined testing and assessments (laboratory testing and physical examinations) that were performed prior to an infusion will not be defined as IRRs.

A list of the most common IRRs that have been reported in patients with Fabry disease during REPLAGAL infusions is included in the current version of the REPLAGAL investigator's brochure.

Management of Infusion-Related Reactions

The management of IRRs should be based on the severity of the reaction, and at the discretion of the investigator. If mild or moderate acute infusion reactions occur, medical attention must be sought immediately, and appropriate actions instituted. The infusion can be temporarily interrupted (5 to 10 minutes) until symptoms subside and the infusion may then be restarted, or the infusion rate can be slowed. Some mild and transient effects may not require medical treatment or discontinuation of the infusion.

Oral or IV pretreatment with antihistamines and/or corticosteroids, from 1 to 24 hours prior to infusion, may prevent subsequent reactions in those cases where symptomatic treatment was previously required. Pretreatment of infusion reactions is left to the clinical judgment of the investigator.

If further analysis of IRRs is necessary, additional clinical laboratory samples may be collected at the discretion of the investigator and in consultation with the study medical monitor.

Symptoms of the Disease under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected disease progression and are part of the efficacy or efficacy data collected in the study. Significant worsening of symptoms should be recorded as an AE.

Preexisting conditions prior to initiation of study medication are described in the medical history, and those that manifest with the same severity, frequency, or duration after drug exposure, are not to be recorded as AEs. However, when there is an increase in the severity, duration or frequency of a preexisting condition, the event must be described in the subject's source documentation.

Clinical Laboratory and Other Safety Assessment

A change in the value of a clinical laboratory parameter, vital sign measure, or ECG assessment can represent an AE if the change is clinically relevant or if, during administration of investigational product, a shift of a parameter is observed from a value in the normative range to a value that is outside the normal range and considered clinically significant, or a further worsening of an already clinically significant value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing administration or after the end of administration with the investigational product, and the range of variation of the respective parameter within its reference range, should also be considered.

If, at the end of the treatment phase, there are abnormal clinical laboratory (such as hematology panel or clinical chemistry panel), vital sign, or ECG values which were not present at the pretreatment evaluation observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease or expected disease evolution) is found for the abnormal values.

The investigator should assess, based on the above criteria and the clinical condition of the subject, whether a change in a clinical laboratory value, vital sign, or ECG parameter is clinically significant and represents an AE.

Appendix 3.2 Collection of Adverse Events

All AEs/SAEs are collected from the time the informed consent document is signed until the defined follow-up period stated in Section 8.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not investigational product is administered.

All AEs/SAEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to baseline), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained.

Appendix 3.3 Assessment of Adverse Events

Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity is captured as a new event. Worsening medical conditions, signs or symptoms present prior to initiation of investigational product, must be recorded as new AEs.

For example, if a subject reports mild intermittent dyspepsia prior to initiation of dosing with the investigational product, and the dyspepsia becomes severe and more frequent after first dose a new AE of severe dyspepsia (with the appropriate date of onset) should be documented in the source.

The medical assessment of severity is determined by using the following definitions:

- **Mild:** A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate:** A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- **Severe:** A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Relationship Categorization

A physician/investigator must make the assessment of relationship to investigational product for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source.

The following additional guidance may be helpful:

Table A1 Adverse Event Relationship Categorization

Related	The temporal relationship between the event and the administration of the investigational product is compelling enough and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the investigational product and the event.

Outcome Categorization

The outcome of AEs must be documented in the source during the course of the study. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- AE Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown.

If applicable, action taken (ie, dose increased, dose not changed, dose reduced, drug interrupted, drug withdrawn, not applicable, or unknown) should also be recorded in the source documentation.

Appendix 3.4 Safety Reporting

Reference Safety Information

The RSI for this study is the investigator's brochure which the sponsor has provided under separate cover to all investigators.

Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the sponsor Global Patient Safety Evaluation (GPSE) Department (see details below) and the sponsor medical monitor within 24 hours of becoming aware of the event. This will be done by transmitting an EDC SAE report, the preferred method of reporting SAEs. If access to EDC is not feasible

within 24 hours of receiving the event, the paper SAE forms should be submitted via fax or email. If a fax is used for transmission, site personnel must make every effort to confirm successful transmission of all pages and include an e-mail address on the fax cover sheet so that an acknowledgment of receipt can be returned via e-mail within 1 business day.

Global Patient Safety Evaluation E-mail: drugsafety@shire.com

Global Patient Safety Evaluation Fax Number: 1-484-595-8155

If e-mail submission of scanned SAE forms is used, site personnel must make every effort to confirm successful transmission by awaiting acknowledgment of receipt via e-mail within 1 business day.

If SAEs are reported via fax or by email, EDC must be updated as soon as possible with the appropriate information. Information in the SAE report form must be consistent with the data provided on the eCRF.

Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Appendix 3.9) unless they result in an SAE.

Appendix 3.5 Serious Adverse Event Collection Time Frame

All SAEs (regardless of relationship to investigational product) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 8.1.4 and must be reported to the sponsor Global Patient Safety Evaluation within 24 hours of the first awareness of the event. Any SAE considered related to study participation that is reported to the investigator following the AE collection period should be reported to the sponsor within 24 hours of first awareness of the event.

Appendix 3.6 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms reported by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

Appendix 3.7 Fatal Outcome

Any SAE that results in the subject's death (eg, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another investigational product action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the investigational product should be recorded as "dose not changed" or "not applicable" (if the subject never received investigational product). The investigational product action of withdrawn should not be selected solely as a result of the subject's death.

Appendix 3.8 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 8.1.4.

Any report of pregnancy for any female study participant must be reported within 24 hours to the sponsor Global Patient Safety Evaluation using the sponsor Pregnancy Report Form.

A copy of the sponsor Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO/sponsor medical monitor using the details specified in the emergency contact information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days postpartum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the SAE eCRF. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the SAE eCRF as well as the Sponsor Pregnancy Report Form. The test date of the first positive serum/urine β-hCG test or ultrasound result will determine the pregnancy onset date.

Appendix 3.9 Abuse, Misuse, Overdose and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Appendix 3.1.

Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of investigational product when used for a nonmedical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- Misuse – Intentional use of investigational product other than as directed or indicated at any dose (Note: this includes a situation where the investigational product is not used as directed at the dose prescribed by the protocol)
- Overdose – Intentional or unintentional intake of a dose of investigational product higher than the protocol-prescribed dose
- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an investigational product. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the investigational product are not considered reportable as medication errors. Medication errors should be collected/reported for all products under investigation. The administration and/or use of an expired investigational product, errors relating to rate of drug administration dilution, use of inappropriate diluent, and longer than recommended time frames within which drug must be used after dilution should be considered as reportable medication errors.

Appendix 3.10 Urgent Safety Measures

An urgent safety measure is an immediate action taken, which is not defined by the protocol, in order to protect subjects participating in a clinical study from immediate harm, these do not constitute de facto deviation from the protocol. Urgent safety measures may be taken by the sponsor or clinical investigator, and may include any of the following:

- Immediate change in study design or study procedures
- Temporary or permanent halt of a given clinical study or studies
- Any other immediate action taken in order to protect clinical study participants from immediate hazard to their health and safety.

The investigator may implement urgent safety measures to protect study subjects from immediate hazard to their health or safety. The measures should implement immediately and does not require prior authorization from the sponsor. In the event(s) of an apparent direct hazard to the subject, the investigator will notify the sponsor immediately by telephone and confirm notification to the sponsor in writing as soon as possible, and within 1 calendar day after the change is implemented. The sponsor will also ensure the responsible EC(s) and relevant competent authority(s) are notified of the urgent safety measures taken in such cases according to local regulations.

Appendix 3.11 Regulatory Agency, Institutional Review Board, Ethics Committee and Site Reporting

The sponsor or designee is responsible for notifying the relevant regulatory authorities of related, unexpected SAEs.

In addition, the sponsor is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the REPLAGAL® program.

The investigator is responsible for notifying the local IRB/EC of SAEs or significant safety findings that occur at his or her site as required by IRB/EC procedures (see Appendix 1.5).

Appendix 4 Contraceptive Guidance

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described below.

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation • Oral • Intravaginal • Transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation • Oral • Injectable
Highly Effective Methods That Are User Independent[a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS). Bilateral tubal occlusion
Vasectomized partner A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the women of childbearing potential (WOCBP) and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
Sexual abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
NOTES: a) Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies.

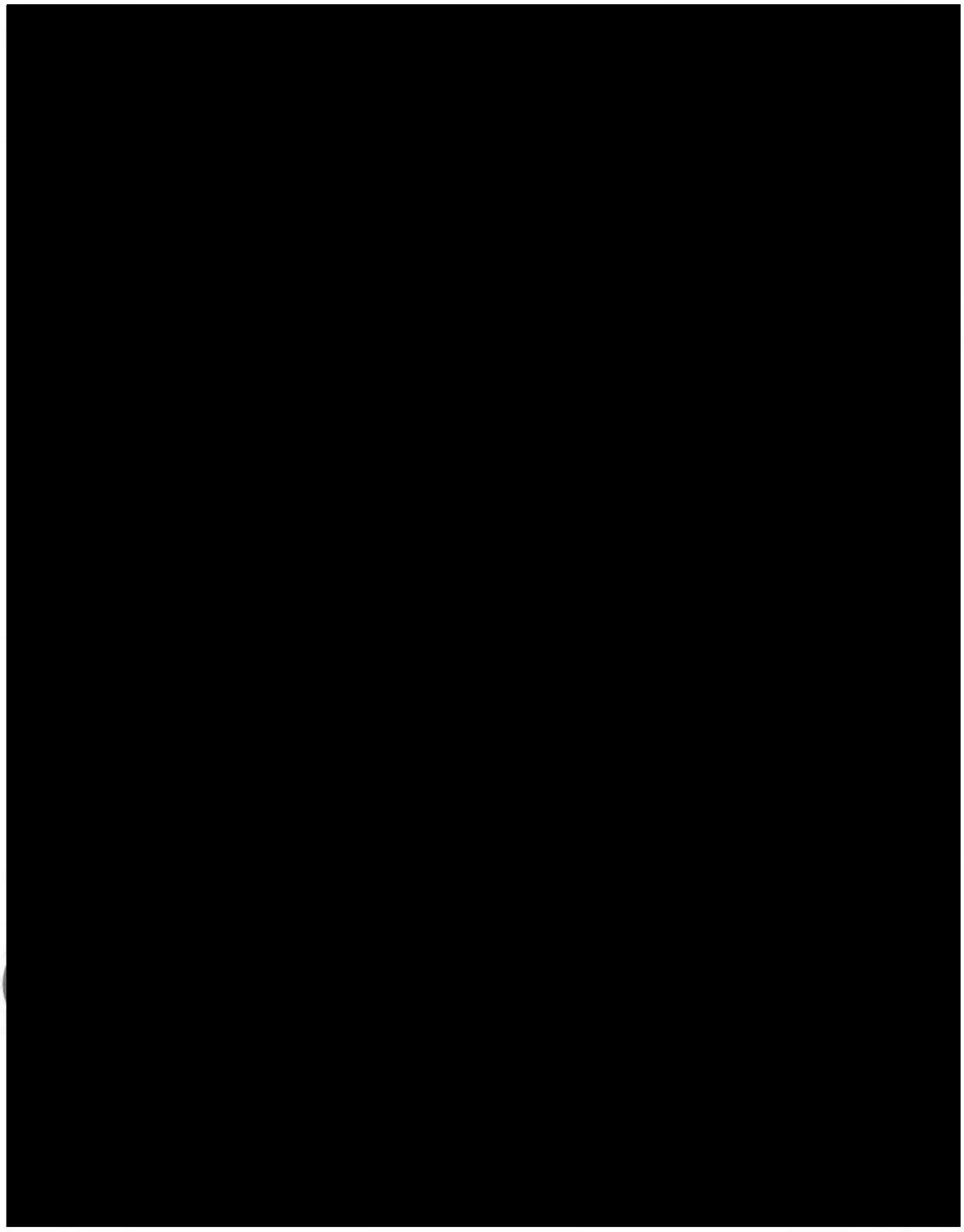
Appendix 5 Scales and Assessments

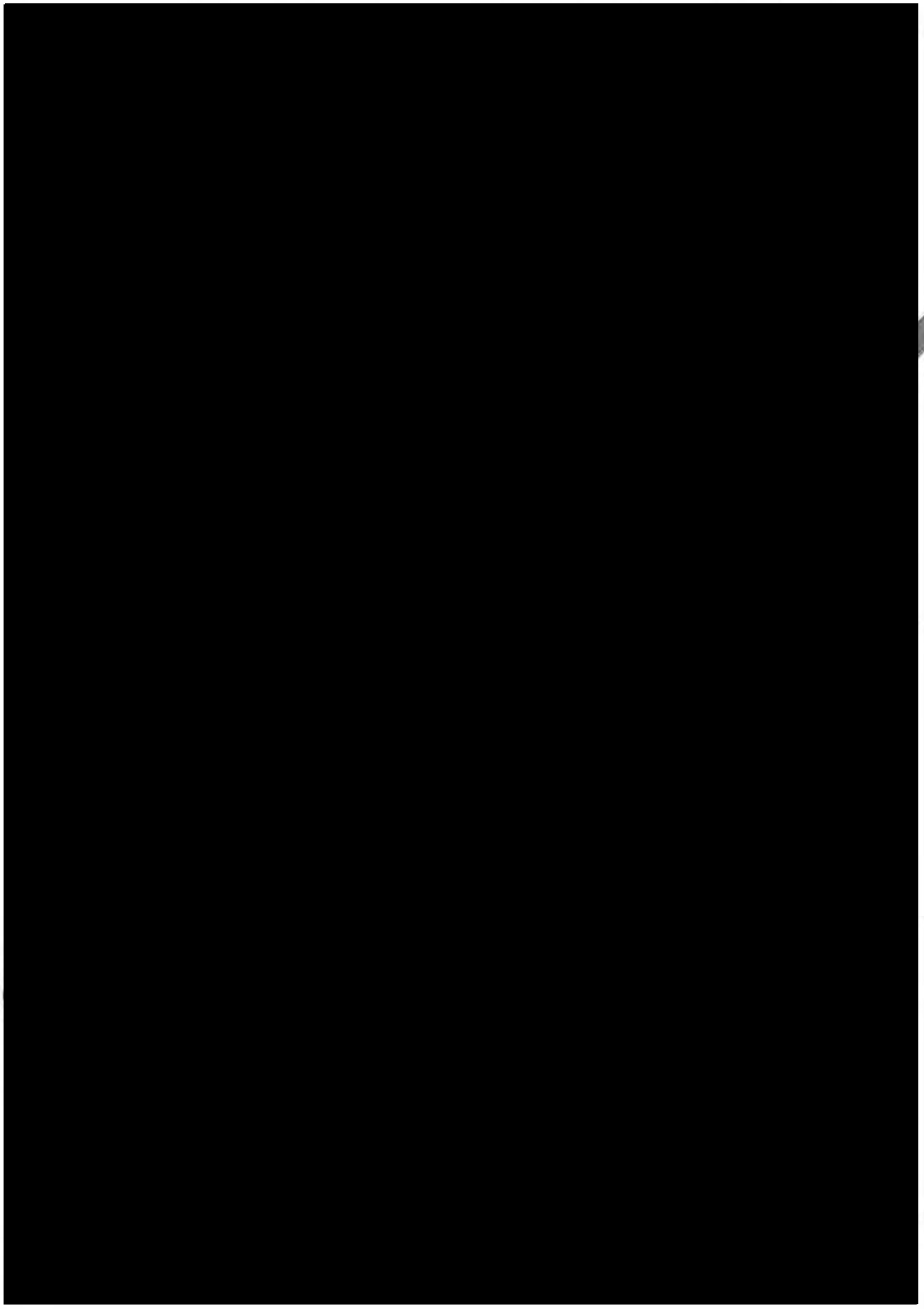
The following scales/assessments will be utilized in this study. In the case of an unplanned event that disallows/prevents the administration of the PRO on a device, for reasons including but not limited to, device outage or other technical limitation, subjects may record their responses on a web back-up version of the assessments that is provided by the CRO or PRO vendor. Sites will be provided with access to the web back-up assessments as a preventative measure.

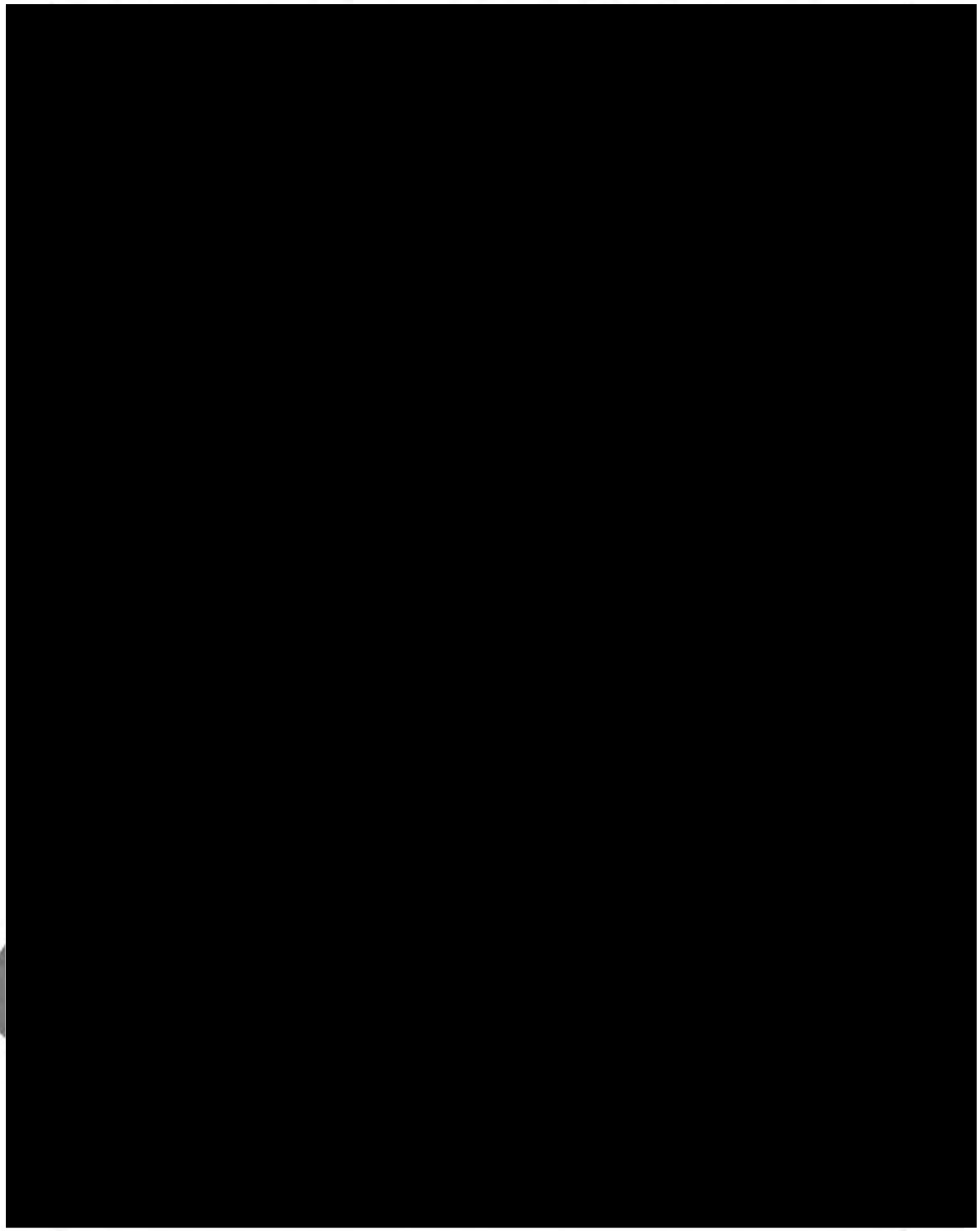
Full Title of Scale/Assessment
Appendix 5.1 Short Form-36 Health Survey (SF-36v2)
Appendix 5.2 Brief Pain Inventory-Short Form (BPI-SF)
Appendix 5.3 Patient Global Impression of Fabry Symptom Severity (PGI-S)
Appendix 5.4 Brief Fatigue Inventory (BFI)
Appendix 5.5 Gastrointestinal Symptom Rating Scale (GSRS)

Appendix 5.1 Short Form-36 Health Survey (SF-36v2)

[redacted]







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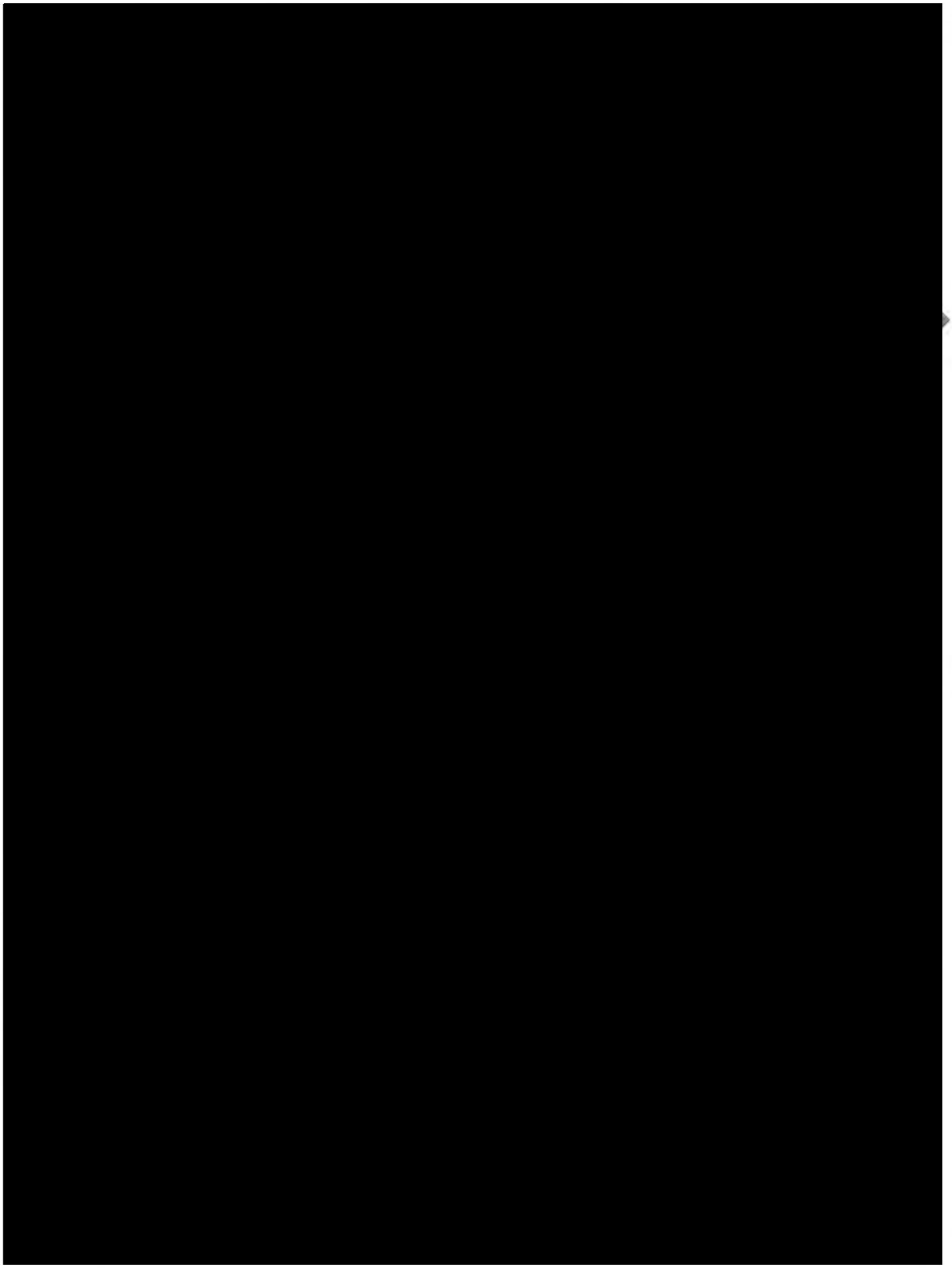

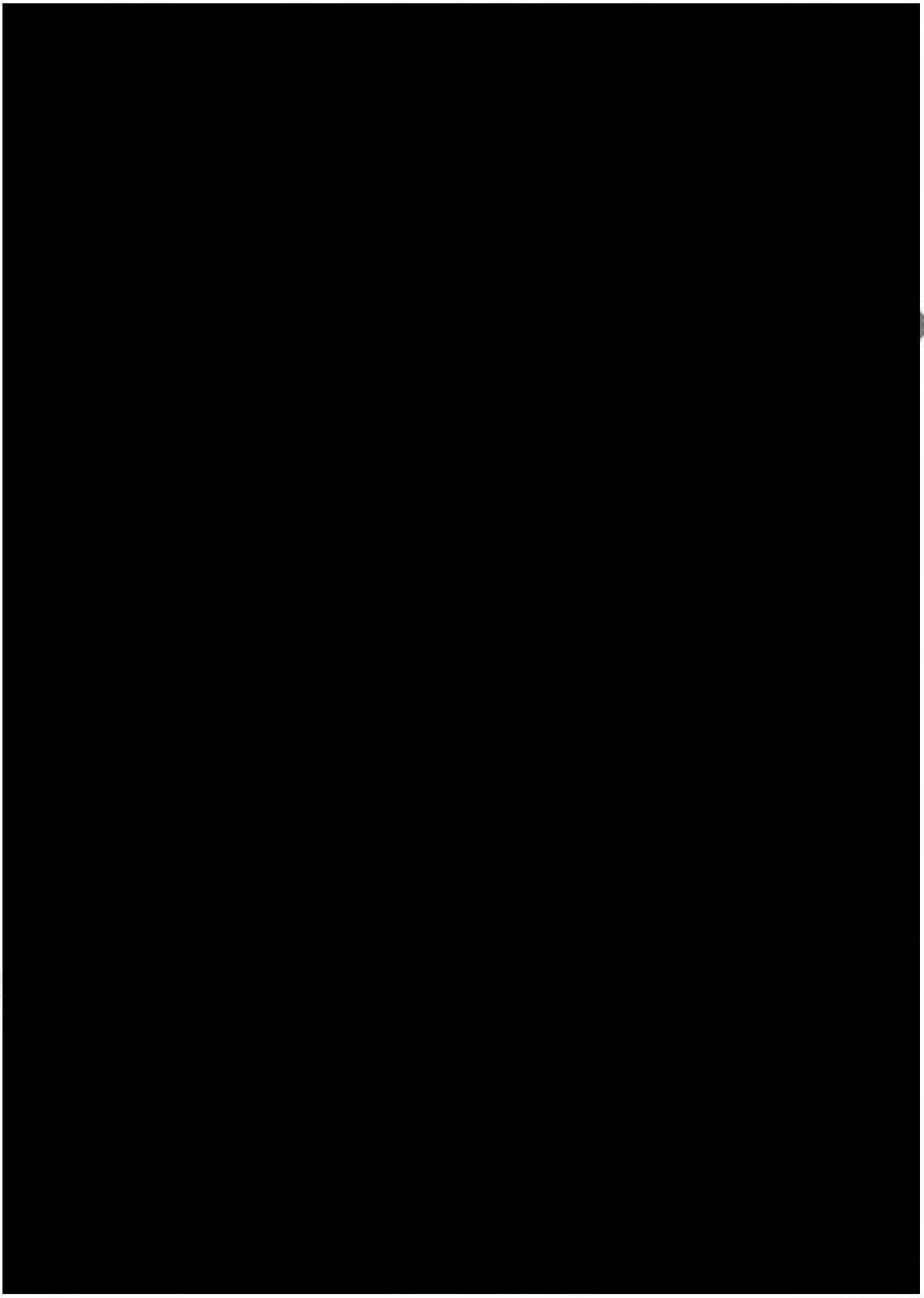

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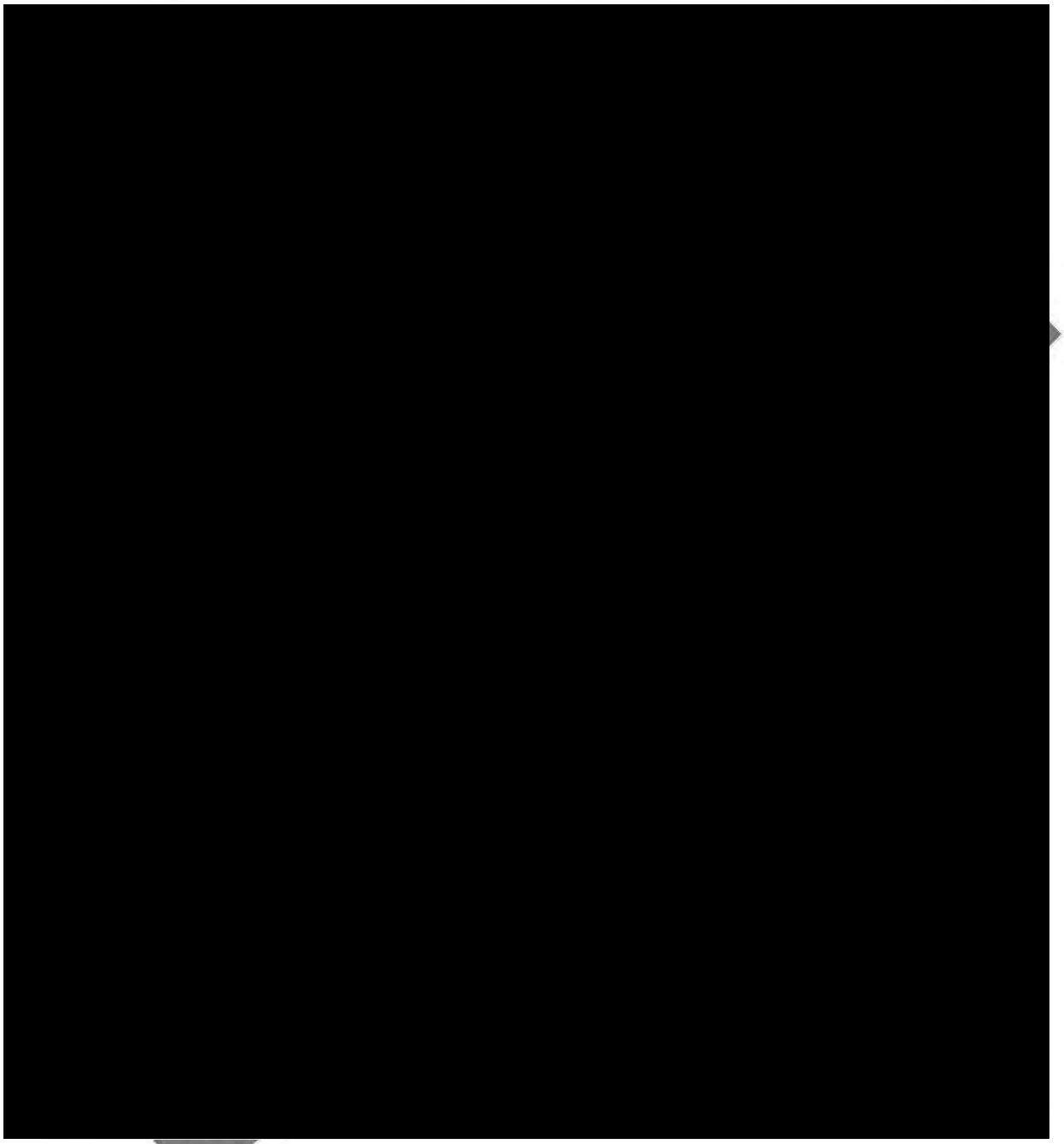

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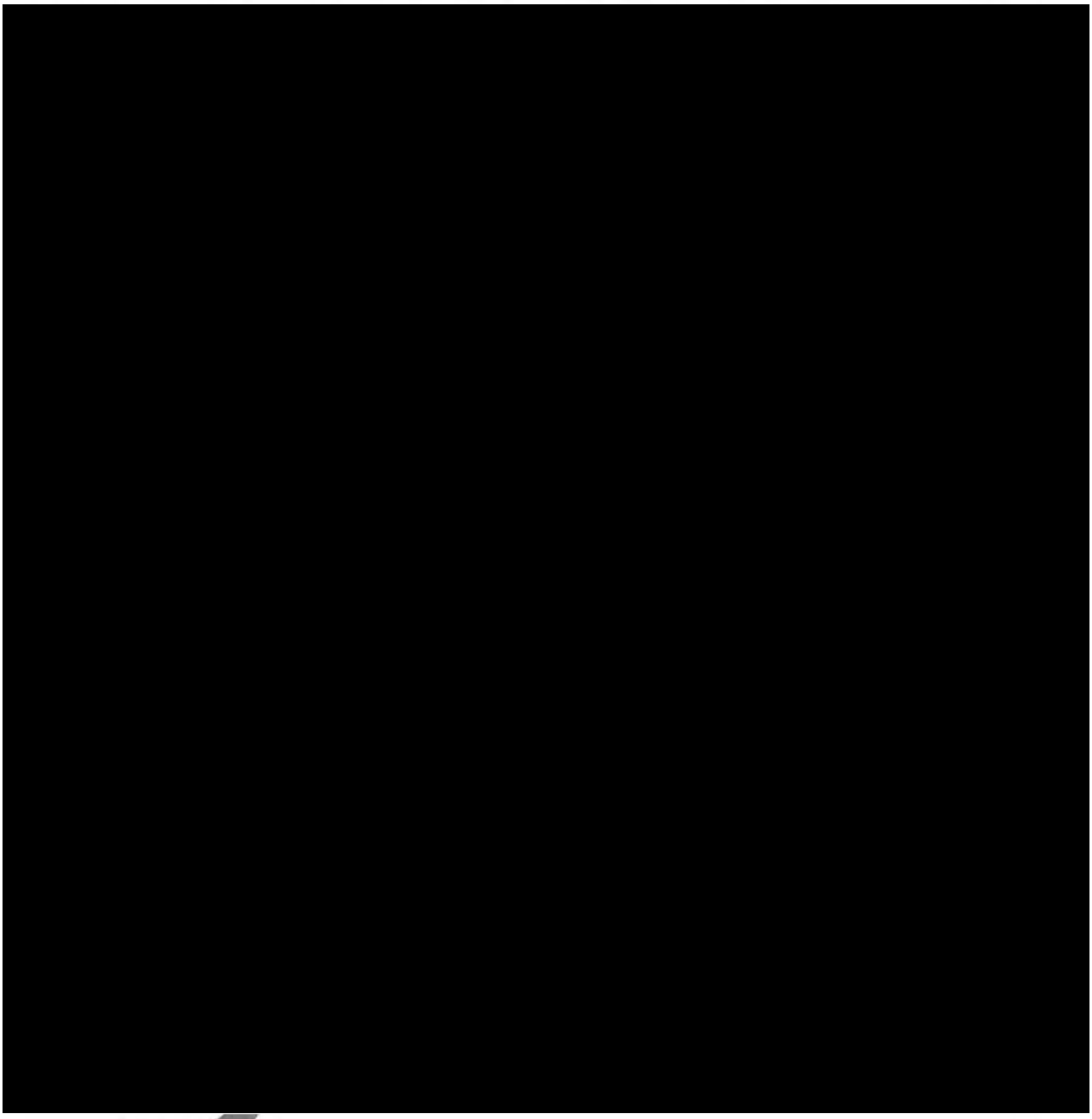

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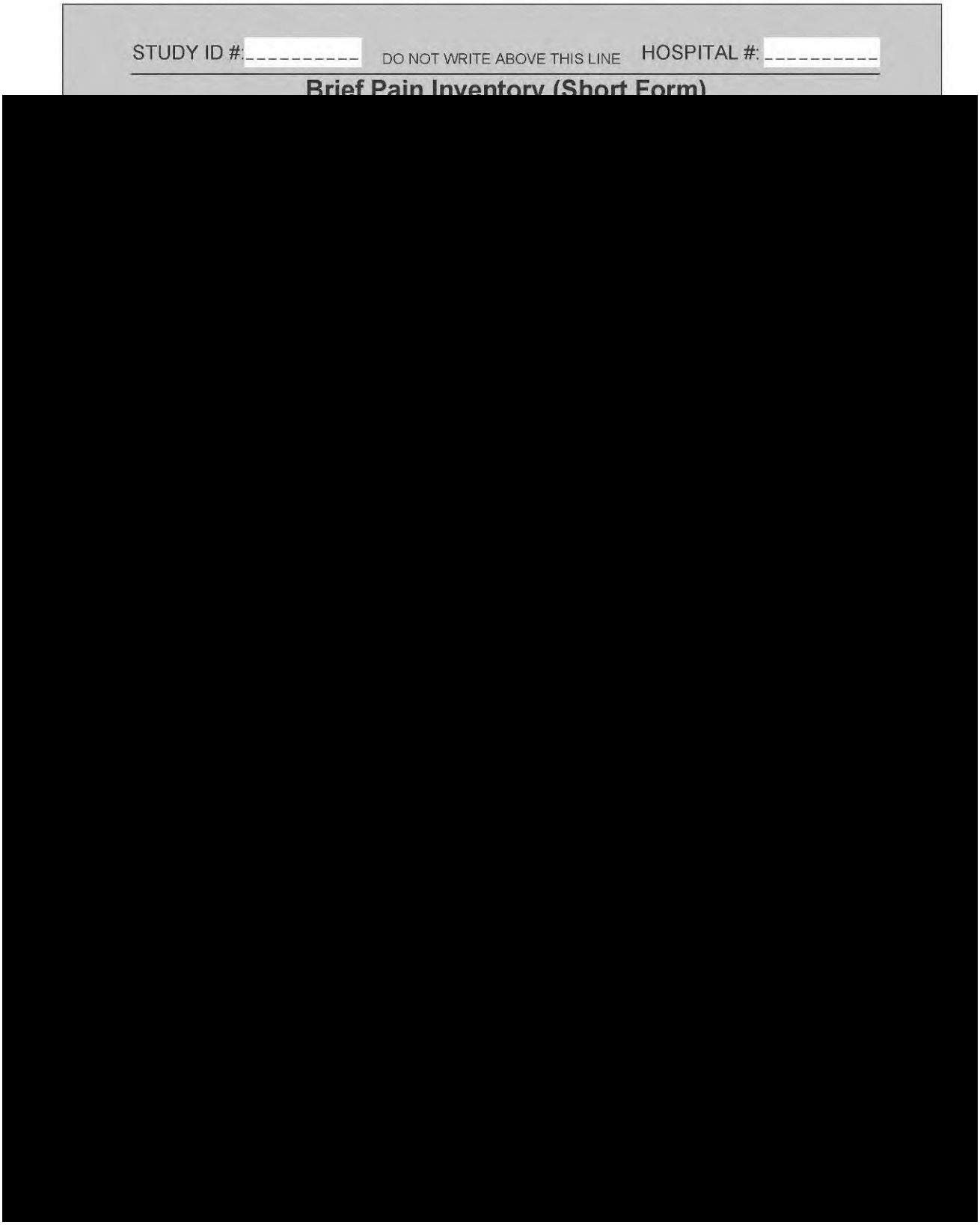
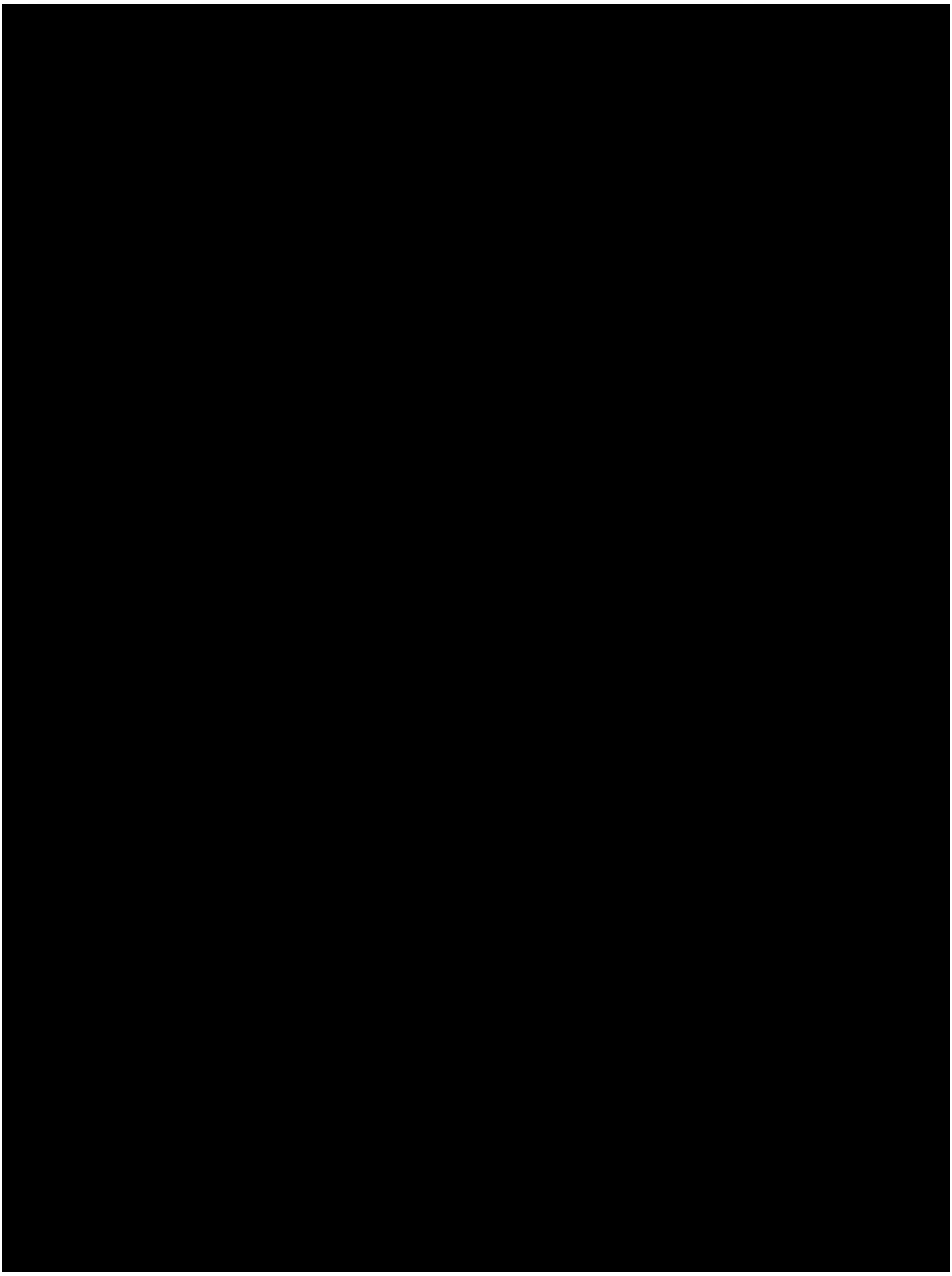
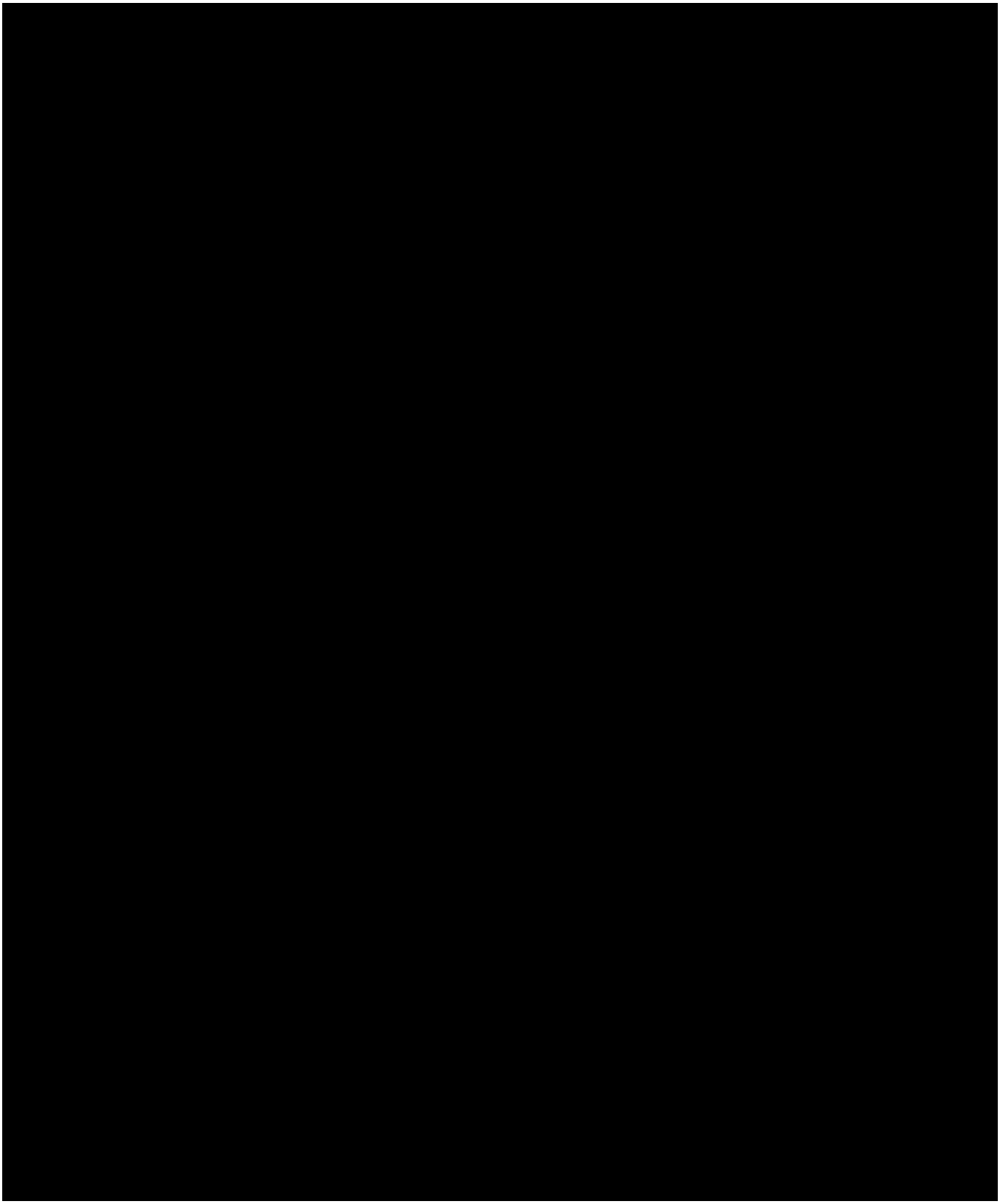
Appendix 5.2 Brief Pain Inventory Short Form (BPI-SF)





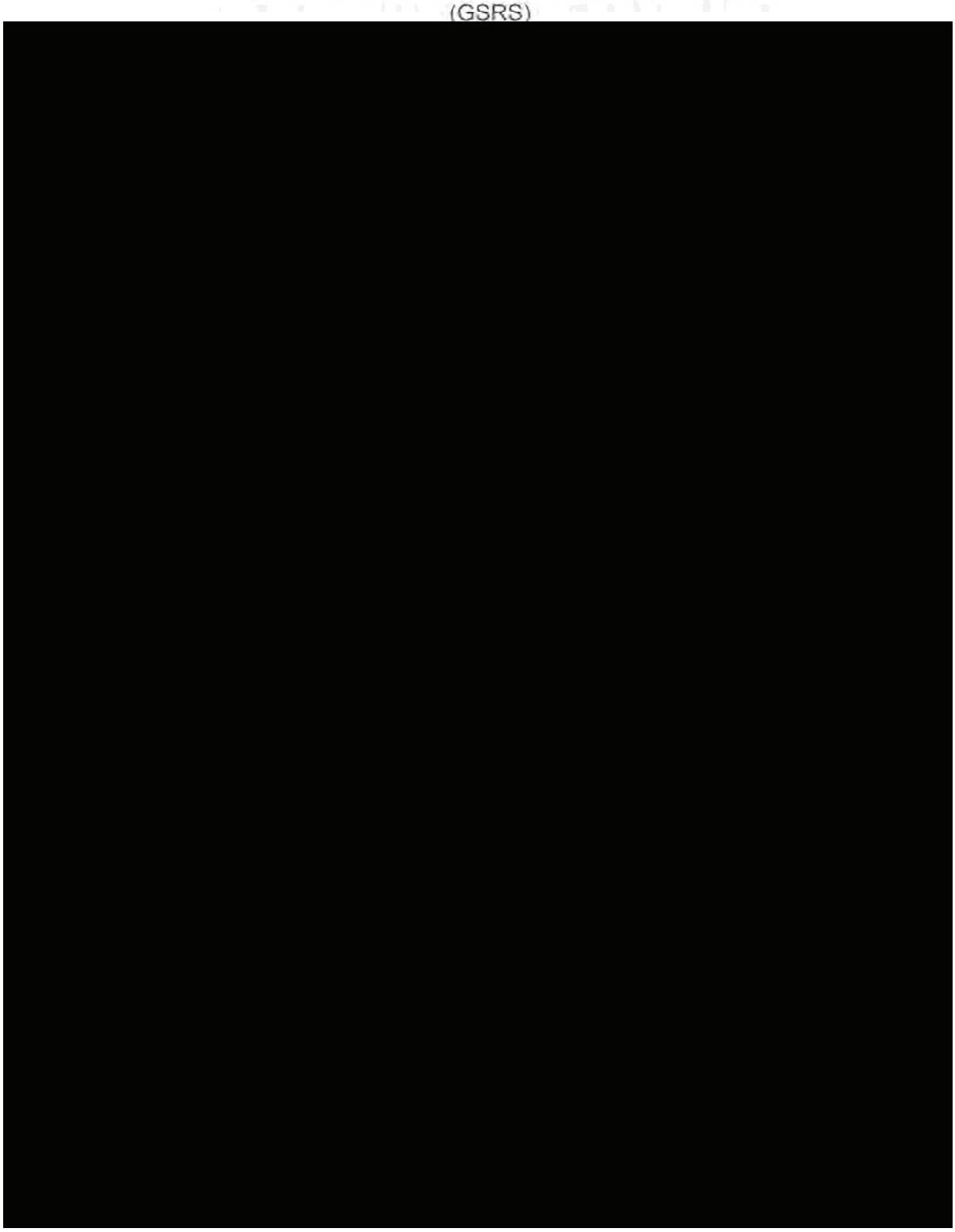
Appendix 5.3 Patient Global Impression of Fabry Symptom Severity (PGI-S)

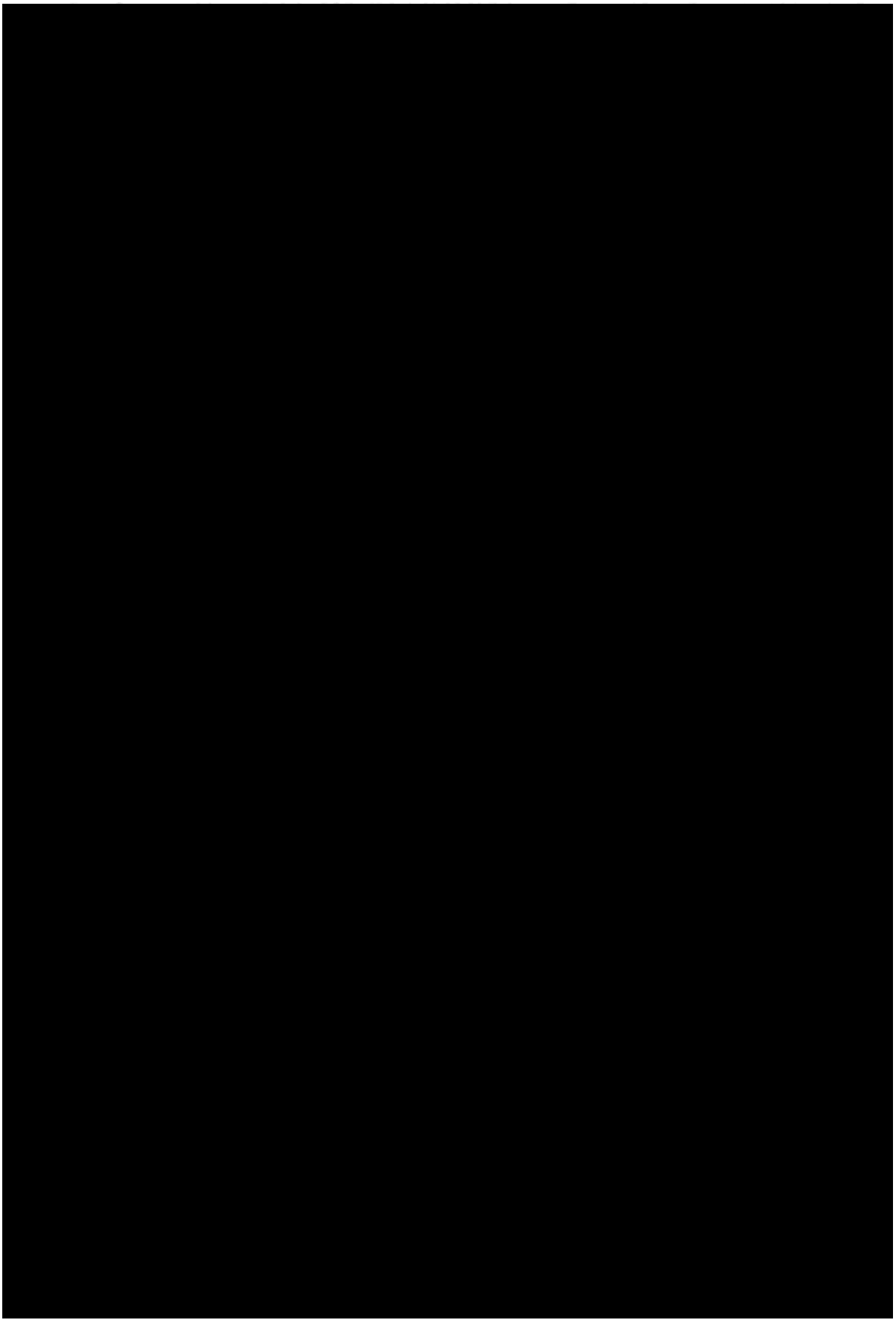
Appendix 5.4 Brief Fatigue Inventory (BFI)

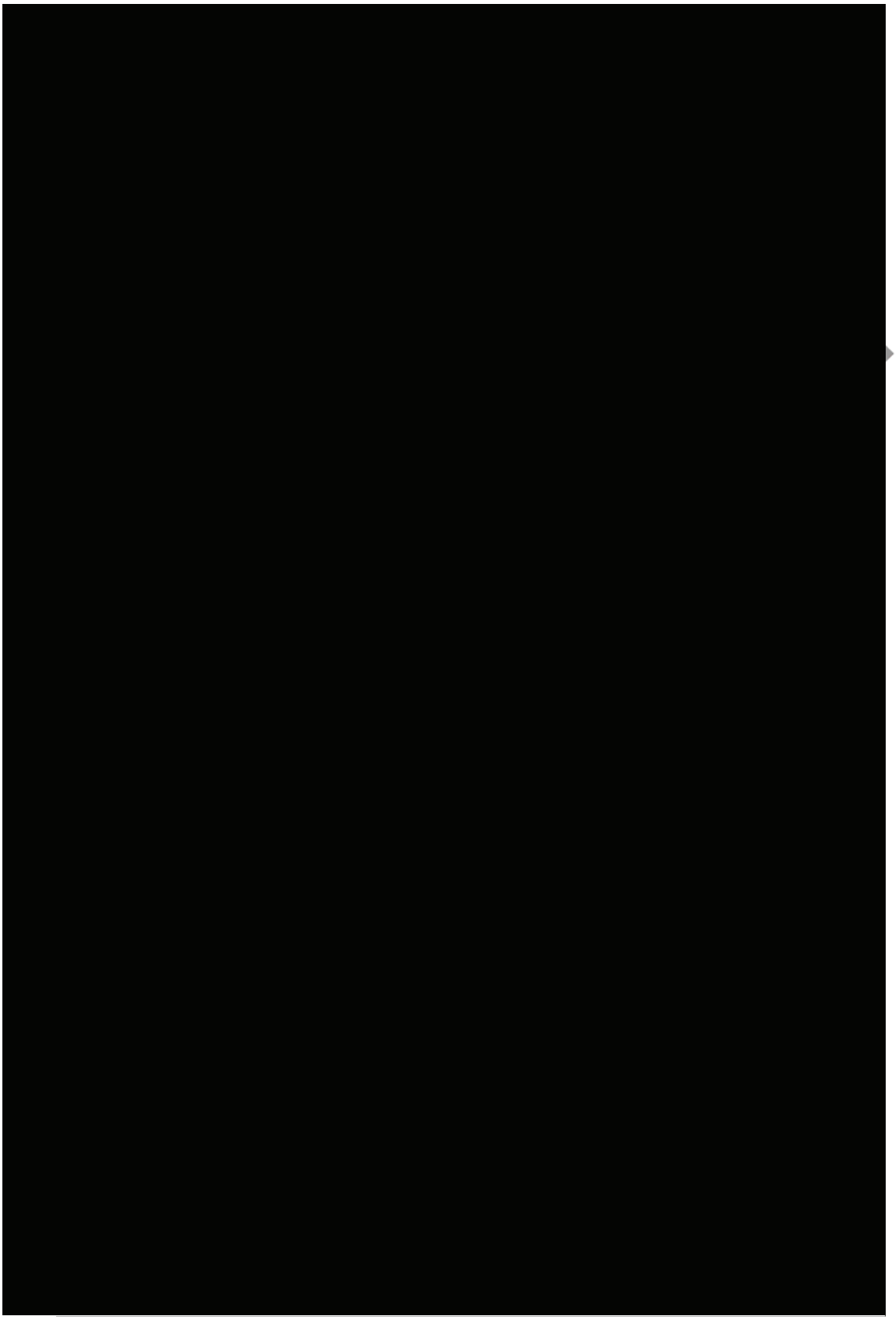


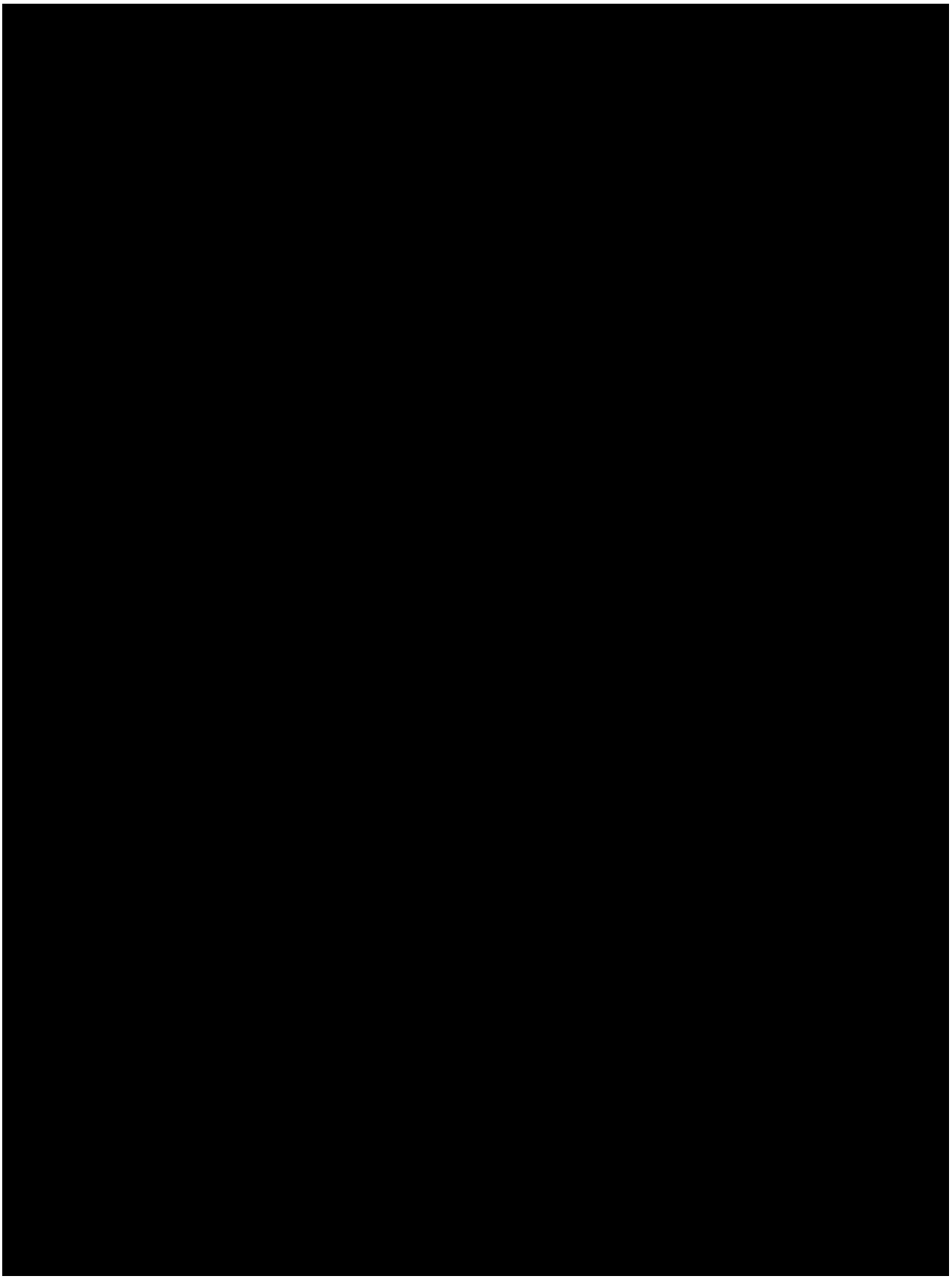
Appendix 5.5 Gastrointestinal Symptom Rating Scale (GSRS)

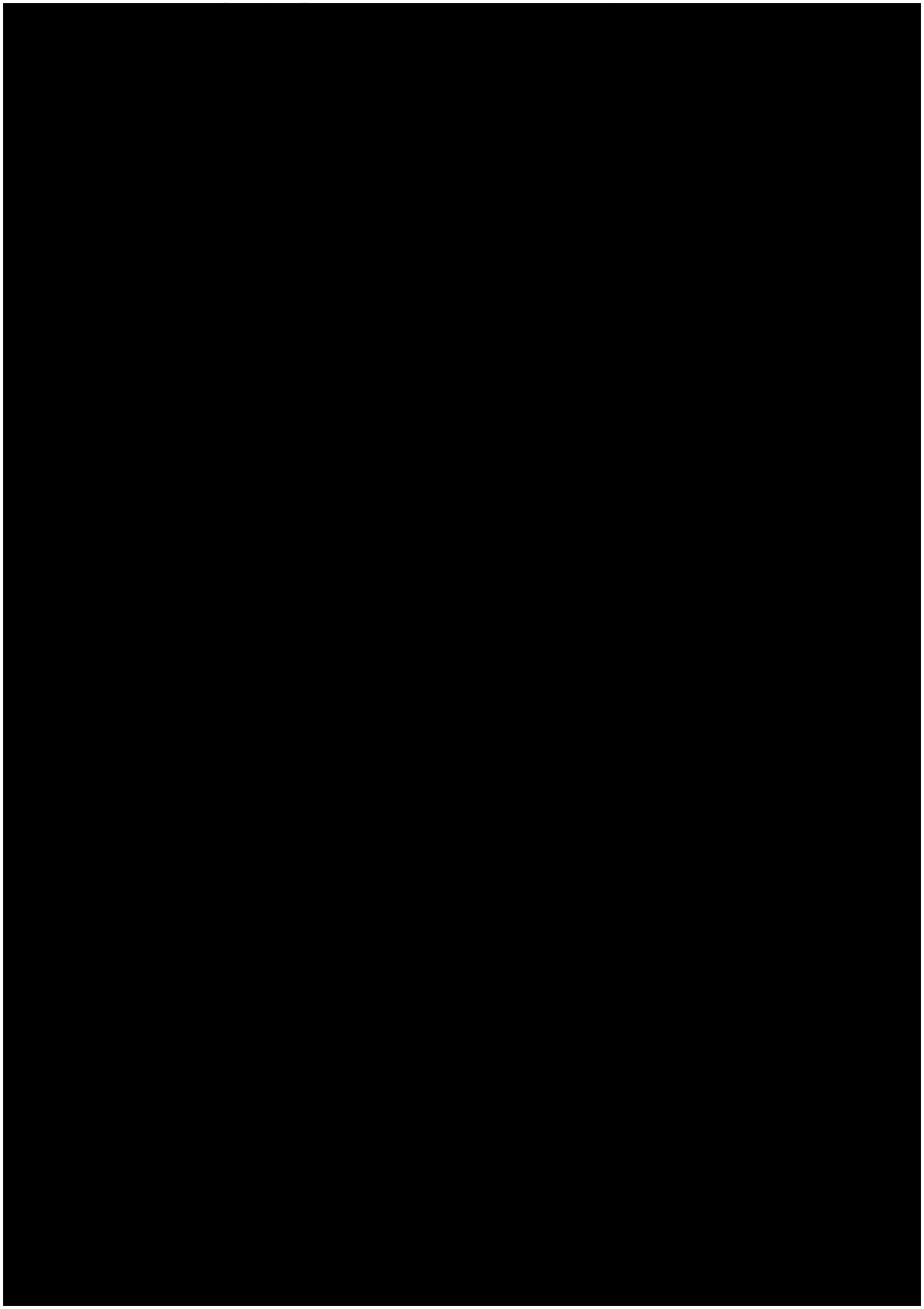
THE GASTROINTESTINAL SYMPTOM RATING SCALE
(GSRS)











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Appendix 6 Abbreviations

Abbreviation	Definition
ADA	antidrug antibody
AE	adverse event
AF	animal-free
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
B19V	parvovirus B19
β-hCG	beta-human chorionic gonadotropin
BFI	Brief Fatigue Inventory
BPI-SF	Brief Pain Inventory-Short Form
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CKD-EPI	Chronic Kidney Disease Epidemiology
CI	confidence interval
CKD	chronic kidney disease
cMRI	cardiac magnetic resonance imaging
CRA	clinical research associate
CRO	contract research organization
CSR	clinical study report
DBS	dried blood spot
EC	ethics committee
ECG	electrocardiography
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
EMA	European Medicines Agency
EOT	end of treatment
EOW	every other week
ERT	enzyme replacement therapy
EU	European Union
FDA	Food and Drug Administration
FOS	Fabry Outcome Survey
Gb_3	globotriaosylceramide
GCP	Good Clinical Practice
GI	gastrointestinal
GLA	α-galactosidase A
GSRS	Gastrointestinal Symptom Rating Scale

Abbreviation	Definition
HAV	hepatitis A virus
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HDL	high-density lipoprotein
HEV	hepatitis E virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
ICAM1	Intercellular Adhesion Molecule 1
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IgE	immunoglobulins E
INTLK1β	Interleukin 1 Beta
IRB	Institutional Review Board
IRR	infusion-related reaction
IRT	interactive response technology
mITT	modified intent-to-treat
IV	intravenous
IVSTd	interventricular septal end-diastolic thickness
KDIGO	Kidney Disease Improving Global Outcomes
LDL	low-density lipoprotein
LVH	left ventricular hypertrophy
LVMI	left ventricular mass index
lyso-Gb3	globotriaosylsphingosine
M6P	mannose-6-phosphate
MI	multiple imputation
MMRM	mixed-effects model for repeated measures
MNAR	missing not at random
NAb	neutralizing antibody
PCR	protein/creatinine ratio
PD	pharmacodynamic
PGI-S	Patient Global Impression of Fabry Symptom Severity
PK	pharmacokinetic(s)
PP	per-protocol
PRO	patient-reported outcome
PWTd	posterior wall thickness in diastole
QoL	quality of life

Abbreviation	Definition
RB	roller bottle
REML	restricted maximum likelihood estimation
RSI	Reference Safety Information
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SF-36v2	Short Form-36 Health Survey
TEAE	Treatment emergent adverse events
TNF-α	Tumor Necrosis Factor
UK	United Kingdom
US	United States
VCAM1	Vascular Cell Adhesion Molecule 1

Appendix 7 Protocol History

Document	Date	Global/Country/Site Specific
Amendment 6	17 Feb 2022	Global
Amendment 5	22 Jul 2021	Global
Amendment 4	24 Jun 2021	Global
Amendment 3	30 Apr 2021	Global
Amendment 2	21 Jan 2021	Global
Amendment 1	01 Apr 2020	Global
Original Protocol	17 Jan 2019	Global

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **01 Apr 2020**	**Global**
Protocol Amendment Summary and Rationale: The protocol was updated to reflect feedback from Health Canada.		
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
All	Made minor updates to text and formatting throughout the protocol	Edited for clarity and consistency
All	Updated "effectiveness" to "efficacy" throughout the protocol	This is a confirmatory study of efficacy, so the term "efficacy" is appropriate
All	Changed time points from Month to Week and adjusted duration of study treatment in weeks	This change corrects study treatment duration from 96 weeks to 104 weeks, which is required in order to have subject treatment data for 2 years
All	Changed "12-week Interval Visits" to "Site Visits"	The study visits are not exactly at 12-week intervals, so "site visits" is more accurate
All	Removed pulse oximetry from vital sign collection	Assessed as not needed
Emergency Contact Information	Updated the emergency contact	New emergency contact
Section 1.1, Synopsis	Updated synopsis to reflect changes in the protocol body	Edited for consistency
Section 1.1, Synopsis	Deleted study design figure	Deleted to reduce the length of the synopsis
Section 1.2, Schedule of Activities Section 3.1.4, Study Endpoints Section 5.2, Exclusion Criteria Section 8.1, Study Periods (and subsections)	Removed the option to use echocardiogram to assess cardiac structure	Left ventricular hypertrophy is now an inclusion criterion, so all subjects must have cardiac magnetic resonance imaging performed; echocardiogram is therefore redundant

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **01 Apr 2020**	**Global**
Protocol Amendment Summary and Rationale: The protocol was updated to reflect feedback from Health Canada.		
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 1.2, Schedule of Activities Section 5.1, Inclusion Criteria Section 8.1, Study Periods (and subsections) Section 8.2.2, Efficacy Section 9.5, Efficacy Analyses (and subsections)	Added that estimated glomerular filtration rate will be calculated by a Shire-designated laboratory	Clarification that this will not be calculated by the site staff
Section 1.2, Schedule of Activities Section 8.1.2, Baseline Visit	Removed proteinuria assessment at baseline	To help alleviate the assessments burden. The screening value will be used so the baseline value would be redundant
Section 1.2, Schedule of Activities Section 8.1.2, Baseline Visit Section 8.1.3, Treatment Period	Removed cMRI from baseline visit and Week 78 and extended the window for screening assessment to 42 days	To help alleviate the assessments burden on subjects and study personnel. Extending the assessment window allows for cMRI screening results to be used for the baseline comparison
Section 1.2, Schedule of Activities Section 8.1.3, Treatment Period	Added Day0/Week 0 to the Schedule of Activities within the Baseline Period to indicate the first infusion of REPLAGAL	Clarifies that the first dose of REPLAGAL will be at Day 0/Week 0 and will be after all baseline assessments and procedures are completed
Section 1.2, Schedule of Activities Section 8.1.1, Screening Period Section 8.1.2, Baseline Period	Moved height, weight, and dose calculation from screening to baseline	To streamline assessments
Section 1.2, Schedule of Activities Section 8.1.3.4, Week 104 Visit	Added a footnote to the EOT visit to indicate all but the REPLAGAL infusion is to be completed at this visit for subjects who discontinue early	Allows for the EOT visit to serve as end of study for all subjects regardless of whether they complete or discontinue the study early
Section 1.2, Schedule of Activities Section 8.2.1.3, Confirmation of Fabry Disease (GLA Activity and Genotyping)	Added GLA activity for female subjects with clarification that this will not be used as an inclusion criterion	For completeness of data
Section 2.2, Product Background and Clinical Information	Deleted data on exposure to commercial REPLAGAL and some text on the efficacy of REPLAGAL	Deletions suggested by Health Canada and in order to ensure the most accurate and current information for safety and efficacy, the latest edition of the REPLAGAL investigator's brochure should be the source referenced

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **01 Apr 2020**	**Global**
Protocol Amendment Summary and Rationale: The protocol was updated to reflect feedback from Health Canada.		
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 4.1, Overall Design Section 7.2, Withdrawal From the Study Section 7.3, Discontinuation of Study Treatment	Added that subjects who discontinue from study treatment will continue to have study visits if they consent to do so	Added in line with guidance from the US National Research Council guidance on The Prevention and Treatment of Missing Data in Clinical Trials
Section 4.1, Study Design Section 9.3, Sample Size and Power Considerations Section 9.4, Statistical Analysis Sets	Updated definition of evaluable subjects to require that subjects must not have discontinued from investigational product	Updated to reflect that data will still be collected from subjects who discontinue treatment but continue study assessments.
Section 4.3, Justification for Dose	Updated the number of countries where REPLAGAL is approved	Updated numbers
Section 6.2.6, Dose Modification Section 8.2.4, Other	Moved dosing information from Section 8.2.4 to Section 6.2.6	Consolidates dosing information in one location
Section 5.1, Inclusion Criteria	Added left ventricular hypertrophy as an inclusion criterion	Added to increase the similarity between the study subjects and the historical control patients per the suggestions from Health Canada
Section 5.2, Exclusion Criteria	Updated exclusion criterion #3 to remove proteinuria >1000 mg/24 hour	Standardized to protein/creatinine ratio to reduce operational burden
Section 5.4.1 Female Contraception Appendix 4, Contraceptive Guidance	Updated contraceptive guidance, including definition of postmenopausal	Updated to reflect most recent protocol template
Section 7.1 Screen Failure	Moved definition of screen failure from Section 8.1.1 to Section 7	Updated for clarity
Section 8.2.5, Volume of Blood to Be Drawn From Each Subject	Increased volume of blood to be taken for antidrug antibody samples from 3 mL to 4 mL, and updated total blood volume accordingly	Volume increased to ensure adequate sample volume for the assay
Section 9.3, Sample Size and Power Considerations	Added details of drop-out rates in previous studies	Justification of the estimated drop-out rate in this study

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **01 Apr 2020**	**Global**
Protocol Amendment Summary and Rationale: The protocol was updated to reflect feedback from Health Canada.		
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 9.3, Sample Size and Power Considerations Section 9.4, Statistical Analysis Sets Section 9.5, Efficacy Analyses (and subsections) Section 9.7.1, Health-related Quality of Life Analyses	Added modified intent-to-treat set	The intent-to-treat set may include subjects who were enrolled in the study but did not receive investigational product. The modified intent-to-treat set will consist of all enrolled subjects who have received at least 1 dose of investigational product and completed at least 1 efficacy assessment of the endpoints being analyzed.
Section 9.7.2, Sensitivity Analysis	New section added	Details how data will be managed regarding sensitivity and outliers
Section 9.8, Missing, Unused, and Spurious Data	Added section on missing, unused, and spurious data	Clarification of how missing data will be treated
Investigator's Responsibilities	Deleted Financial Disclosure information	This is a non-IND study and is not being conducted at any US sites
Data Management Considerations	Added that patient-facing assessments including the SF-36, BPI-SF, PGI-S, BFI, and GSRS will be collected via an electronic tablet	This helps to clarify data collection for these assessments
Adverse Event Definitions	Deleted suggestion that electrocardiograms should be performed in triplicate and conducted by a central laboratory	Electrocardiograms will not be done by a central laboratory
Appendix 5, Scales and Assessments	Updated sample scales and assessments	Updated to provide the most recent versions
Section 10, References	Moved Section up from 11 to 10	Updated to current submission standards
Section 11, Supporting Documentation and Operational Considerations	Moved Section down from 10 to 11	Updated to current submission standards

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 2	**Amendment Date 21 Jan 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Title Page	The title page was updated to state that Shire is now a wholly owned subsidiary of Takeda Pharmaceutical Company Limited.	This change was made to provide updated and accurate information for the sponsor.
Protocol Signature Page, Emergency Contact Information	The medical monitor information for this study was updated as follows: [redacted] Marketed Products.	This change was made to provide updated and accurate information.
Emergency Contact Information, Section 6.2.1, Section 8.2.3.7, Appendix 3.4, Appendix 3.5, Appendix 3.8	The name of the Global Drug Safety department was updated to Global Patient Safety Evaluation.	This change was made to provide updated and accurate information.
Section 1.1 Synopsis, Section 3.1.1, Section 3.2	The primary objective was updated to indicate that efficacy will be evaluated every other week (EOW) for up to 104 weeks.	This change was made to provide updated and accurate information.
Section 1.1 Synopsis, Section 4.5	Sites and Regions have been updated to indicate that the study is expected to be conducted in up to 50 sites globally.	This change was made to provide updated and accurate information.
Section 1.1 Synopsis, Section 4.4	The planned study duration of approximately 6 years has been updated to indicate the first subject enrolled is anticipated in 2021 and the last subject completed is anticipated to be in 2027.	This change was made to provide updated and accurate information.
Section 1.1 Synopsis, Section 4.1, Section 4.2, Section 9.3	Updates have been made to indicate that the study will use reference values of patients with Fabry disease from published literature instead of historical controls.	Historical controls are not available for this study and reference values from published literature will be used.
Section 1.1 Synopsis, Section 5.1	Inclusion criterion 3 was revised as follows: "The subject is 18 to 65 years of age, inclusive."	This change was made because the upper limit of age for inclusion as REPLAGAL has not been tested in subjects over 65 years of age.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **2**	**Amendment Date** **21 Jan 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 1.1 Synopsis, Section 5.2	Exclusion criterion #4 was revised as follows: "Subjects who have clinically relevant history of allergy or signs or symptoms of severe hypersensitivity **(**including hypersensitivity to the REPLAGAL active substance or any of the excipients**),** which in the investigator's judgment, will substantially increase the subject's risk if he or she participates the study."	This change was made to ensure that patients with hypersensitivity to any components in REPLAGAL are not enrolled.
Section 1.1 Synopsis, Section 5.2, Section 6.6.3, Table 1 Schedule of Activities footnote v	Content from exclusion criteria (formerly 10 and 11) 9 and 10 as added to Section 6.6.3 Prohibited Treatment and footnote v in Table 1 Schedule of Activities; these 2 exclusion criteria were combined into a single exclusion criterion.	This content was combined to streamline the eligibility and was also added to the prohibited treatments and medications and footnote "v" in Table 1 for consistency.
Section 1.1 Synopsis, Section 9.3	The details for sample size and power considerations was revised to read: "The null hypothesis for eGFR is that there is no difference in mean change from baseline in eGFR at Week 104 between the treated subjects and the untreated patients~~~~ reference change of -6 mL/min/1.73 m^2. The alternative hypothesis states that the mean decline in eGFR is smaller than this reference value. The null hypothesis for LVMI is that there is no difference in mean change from baseline in LVMI at Week 104 between the treated subjects and the untreated patients~~~~ reference change of +10 $g/m^{2.7}$. The alternative hypothesis states that mean increase in LVMI is smaller than this reference value. The Hochberg procedure to control the type I error rate for the 2 primary endpoints is incorporated in the sample size determination. The Hochberg procedure does not require the order of testing. Below is the sample size justification assuming	The details have been modified to align with the new approach to statistical analysis.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 2	**Amendment Date 21 Jan 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
	only one of the dual endpoints will be determined to be significant. If both endpoints are significant at the 0.025 significance level, then the power will be large than quoted below. For eGFR, with 36 evaluable subjects, the study has at least 90% power to detect a decrease after 104 weeks of treatment of 1.7 mL/min/1.73 m^2 compared with a reference untreated change of -6 mL/min/1.73 m^2 with SD=7 (effect size of 0.61) using a 1-sided one sample t-test at significance level of 0.0125. For LVMI, with 36 evaluable subjects, the study has at least 90% power to detect an increase after 104 weeks of treatment of 0.64 $g/m^{2.7}$ compared with a reference untreated change of 10 $g/m^{2.7}$ with SD=15 (effect size of 0.62) using a 1-sided one sample t-test at significance level of 0.0125."	
Section 1.1 Synopsis, Section 9.4, Section 9.5.1, Section 9.5.2	The intent to treat (ITT) population was removed and the modified intent to treat (mITT) population was revised. The mixed-effects model for repeated measures (MMRM) will use the mITT population for both primary and secondary analyses.	Revised to ensure the subject receives at least one dose of investigational product so that data is more meaningful.
Section 1.1 Synopsis, Section 9.4	A new subject population was added: the completers set	This subject population was added to ensure analysis can be conducted for a larger proportion of the subject population.
Section 1.1 Synopsis, Section 9.5.1, Section 9.5.2	The following was added to the sensitivity analysis: "The eGFR and LVMI annualized rate of change and 95% CI will be estimated for subjects using the random intercept and slope model. Baseline eGFR or baseline LVMI, age at baseline, sex, and treatment duration on investigational product in year will be included in the model."	Statistically it is more appropriate to use this information in the sensitivity analysis.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 2	**Amendment Date 21 Jan 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 1.1 Synopsis, Section 9.5.1	The analyses of the primary endpoints were revised to indicate that the most appropriate data (vs all available data) will be used.	This is a clarification to indicate analysis will be conducted with the most appropriate data.
Table 1 Schedule of Activities	Proteinuria was added to baseline as an assessment to be completed.	This was removed in amendment 1 to ease subject burden but is reconsidered to be important to the study.
Table 1 Schedule of Activities footnote q, Section 8.1.1, Section 8.1.2, Section 8.1.3.2	Details of the complete blood count assessment were added.	Added for completeness and clarity
Table 1 Schedule of Activities	Schedule of Activities Table revised to correct visit windows and clarify site visits	Added for clarity
Section 2.1, Section 10	New statements added with new reference publications: "The threshold level of alpha-Gal A activity below which clinically significant Fabry disease occurs is thought to be 30 to 35 percent of the mean normal control Schiffmann et al., 2016." And "In females, alpha-Gal A activity is most often higher than in males Arends et al., 2017."	To enhance information provided about the indication under study.
Section 2.2	New information on infusion-related reactions (IRRs) was added including the description of the observed events' onset, timing, mitigation, and treatment. Newly added information on IRRs is analyzed in the context of benefit/risk which still remains positive. A summary of the benefit/risk conclusions was added. A statement was added that investigators will follow site-specific standards for the mitigation of impact from COVID-19 and will manage any AEs related to COVID-19 per study procedures for the reporting and treatment of AEs.	Added to inform on observed IRRs, the most commonly observed ADRs associated with REPLAGAL, and to provide detailed guidance on mitigation, potential causes, and treatment. Conclusions and rationale added to confirm benefit/risk is still favorable. Statement added to inform that investigators will follow site-specific standards and AE reporting if there is impact from COVID-19.
Section 6.2.1	A statement that home infusions are optional and not mandatory was added.	To provide clarity.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 2	**Amendment Date 21 Jan 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 6.2.1	Each EOW Infusion mentioned in this section was updated to include the corresponding infusion number.	To provide clarity.
Section 6.2.3, Section 8.2.4.3, Appendix 5	A statement that patient-reported outcome (PRO) assessments may be completed via electronic device or provided paper assessment was added.	Added as a preventative measure in the event that there are technology issues preventing electronic devise use or if the subject is unable to travel to the site.
Section 6.7	A new section was added for COVID-19-related protocol considerations.	Added to ensure subject safety and maintain study data integrity due to any impact due to the COVID-19 pandemic.
Section 7.3	The content for discontinuation of study treatment was expanded and new study stopping rules were added.	Additional detail added for clarification and enhancement of safety precautions.
Section 8.1.2	An assessment was added: "Calculate the dose of REPLAGAL based on the subject's weight at baseline."	This assessment was inadvertently omitted from the description of stud assessments and procedures performed at baseline in the prior amendment.
Section 8.2.1.2	The following text was added: "If the eGFR measurement at screening is not within the range, a second eGFR measurement may be completed and, if in the stipulated range, used as the screening value. If a second measurement is taken, a minimum of 1 week and maximum of 30 days should separate it from the first measurement."	This text was already in inclusion criterion #8 and screening sections and has been added to the section on eligibility to confirm allowance and for consistency.
Section 9.7.1	The assessment of GSRS was added.	These assessments were inadvertently omitted from the prior amendment.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 3	**Amendment Date 30 April 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 1.1	The analyses of the primary endpoints were revised to indicate that all available	Takeda acknowledges that the revised wording in the protocol amendment 2

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number 3	**Amendment Date 30 April 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 9.5.1	data (vs most appropriate data) will be used.	from "all available data" to "the most appropriate data" could alter the interpretation of the statement. This change was an oversight during editorial review of the protocol and was not intended to suggest a change in the approach to the assessment of primary endpoints.
Table 1	For all subjects 18 to 65 years inclusive: height and weight must be collected at Screening. Height must also be measured and collected at Week 52.	Subject height and weight must be collected at Screening, Week 52 and Week 104 (or early termination) visits as required for Cardiac MRI procedure.
Section 8.1.1.1	For all Subjects 18 to 65 years inclusive height and weight are measured at Screening.	Subject height and weight must be measured at Screening as required for Cardiac MRI procedure.
Section 8.1.2	Remove "Measure height" at Baseline.	Subject height must be measured and collected at Screening as required for Cardiac MRI procedure.
Table 1 Section 8.1.3.3 Section 8.1.3.4 Section 8.2.3.2	Subject height must be measured at Screening, Week 52 and Week 104 (or early termination) visits for all subjects.	Subject height must be measured at Screening, Week 52 and Week 104 (or early termination) for all subjects as required for Cardiac MRI procedure.
Table 1 Section 8.2.3.3	Subject weight must be measured at Screening.	Subject weight must be measured at Screening as required for Cardiac MRI procedure.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **4**	**Amendment Date** **24 June 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Synopsis Section 9.4, Statistical Analysis Sets	An intent-to-treat (ITT) subject population was added. The safety set, per protocol set, and completers set will be drawn from the ITT set instead of the mITT set.	The analysis will be based on the ITT population to include all enrolled patients.
Synopsis Section 9.3 Sample Size and Power Considerations Section 9.5.1, Primary Efficacy Endpoints Section 9.5.3 Multiplicity Adjustment Section 9.5.4, Control of Type I Error	Hochberg procedure replaced by Bonferroni adjustment, ITT set instead of mITT set, the primary null hypothesis was updated to include the mean change from baseline in eGFR at Week 104 for treated subjects will be less than or equal to -6 mL/min/1.73 m^2, the reference value from untreated subjects (instead of no difference), and the alternate hypothesis is that the mean change in eGFR is greater than -6 mL/min/1.73 m^2, the reference value (instead of change in absolute value) from untreated subjects. The mean change from baseline in LVMI at Week 104 of treated subjects will be greater than or equal to 10 $g/m^{2.7}$, the reference value from untreated subjects (instead of no difference), and the alternate hypothesis is that the mean change in LVMI will be less than 10 $g/m^{2.7}$, the reference value (instead of change in absolute value) from untreated subjects. A tipping point analysis was added for the ITT and mITT sets Addition of ITT analysis set to analyses	Changes to the statistical analysis sections were made pursuant to a request from a regulatory authority to define the limits used to test the corresponding null hypotheses for the 2 primary endpoints (change from baseline at Week 104 below or above specific limit)
Section 4.2, Scientific Rationale for the Reference Controlled Study Design	Reference data clarified from "historical control" to "natural history" patients	Clarification of source data
Section 9.1	Text added to specify the timing of the SAP added.	The first version of the SAP will be finalized before the enrollment of the first patient.

Protocol Amendment		
Summary of Change(s) Since the Last Version of the Approved Protocol		
Amendment Number **5**	**Amendment Date** **22 July 2021**	**Global**
Section(s) Affected by Change	**Description of Each Change**	**Rationale for Change**
Section 1.1, Synopsis- Statistical Methodology for the Primary Efficacy Endpoints Section 9.5 Efficacy Analysis Section 9.5.1 Primary Efficacy Endpoint	Includes information regarding statistical method for handling missing estimated glomerular filtration rate (eGFR) and left ventricular mass index (LVMI) data.	Clarification of methods for missing eGFR and LVMI data.
Appendix 6	Includes abbreviation for assumption that the missing data is not at random (MNAR).	