Lucerastat / ACT-434964

Fabry disease

Protocol ID-069A301

MODIFY

A Multicenter, dOuble-blind, ranDomized, placebo-controlled, parallel-group study to determine the effIcacy and safety of lucerastat oral monotherapy in adult subjects with FabrY disease.

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SPONSOR CONTACT DETAILS

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Medical Emergency Hotline Toll phone number:	Site-specific toll telephone numbers and toll-free numbers for the Medical Emergency Hotline can be found in the Investigator Site File.

COORDINATING INVESTIGATOR

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CONTRACT RESEARCH ORGANIZATIONS INFORMATION

Some study activities will be delegated to Contract Research Organizations (CROs). A list of site-specific contact details can be found in the Investigator Site File.

SIGNATURE PAGE FOR IDORSIA PHARMACEUTICALS LTD

Hereinafter called Idorsia or sponsor

Treatment name / number

Lucerastat / ACT-434964

Indication

Fabry disease

Protocol number, study acronym, study title

ID-069A301, MODIFY

A multicenter, double-blind, randomized, placebo-controlled, parallel-group study to determine the efficacy and safety of lucerastat oral monotherapy in adult subjects with Fabry disease.

I approve the terms and conditions relating to this study as defined in this protocol. I confirm that the information contained in this protocol is consistent with the current risk-benefit evaluation of lucerastat, and with the ethical and scientific principles governing clinical research as set out in the Declaration of Helsinki and the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines.

Title	Name	Date	Signature
Director, Clinical Project Physician	[redacted] MD	[redacted]	[redacted]

INVESTIGATOR SIGNATURE PAGE

Treatment name / number

Lucerastat / ACT-434964

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A multicenter, double-blind, randomized, placebo-controlled, parallel-group study to determine the efficacy and safety of lucerastat oral monotherapy in adult subjects with Fabry disease.

I agree to the terms and conditions relating to this study as defined in this protocol and any other protocol-related documents. I fully understand that any changes instituted by the investigator(s) without previous agreement with the sponsor would constitute a protocol deviation, including any ancillary studies or procedures performed on study subjects (other than those procedures necessary for the well-being of the subjects).

I agree to conduct this study in accordance with the Declaration of Helsinki principles, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, and applicable regulations and laws.

Principal Investigator	Country	Site number	Town	Date	Signature

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α-GalA	α-galactosidase A
ACE	Angiotensin-converting enzyme
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ARB	Angiotensin receptor blocker
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC_{τ}	Area under the plasma concentration-time curve during one dosing interval
AUC_{0-48}	Area under the plasma concentration-time curve from zero to 48 h after dosing
$AUC_{0-\infty}$	Area under the plasma concentration-time curve extrapolated to infinity
AUC_{0-t}	Area under the plasma concentration-time curve from zero to time t of the last measured concentration above the limit of quantification
b.i.d.	Twice daily
BLQ	Below the limit of quantification
BPI	Brief Pain Inventory
BPI-SF	Brief Pain Inventory-Short Form
BPI-SF3/5/9	Brief Pain Inventory-Short Form item 3/5/9
BSS	Bristol Stool Scale
CESD-R-20	Center for Epidemiologic Studies Depression Scale Revised
CFR	Code of Federal Regulations (US)
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	Maximum observed plasma concentration
CMH	Cochran-Mantel-Haenszel
COVID-19	Coronavirus disease 2019
CR	Copy Reference

CRA	Clinical Research Associate
CRO	Contract Research Organization
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CTFG	Clinical Trials Facilitation Group
CTT	Clinical Trial Team
DBP	Diastolic blood pressure
DDI	Drug-drug interaction
DRG	Dorsal root ganglia
EC	Ethics Committee
eCDF	Empirical cumulative distribution function
ECG	Electrocardiogra(m/phy)
eCRF	Electronic case report form
eDiary	Electronic diary
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EOS	End-of-Study
EOT	End-of-Treatment
ePDF	Empirical probability density function
ERT	Enzyme replacement therapy
EU	European Union
FAS	Full Analysis Set
FD	Fabry disease (Anderson-Fabry disease)
FDA	Food and Drug Administration
FOS	Fabry Outcome Survey
FU	Follow-up
Gb3	Globotriaosylceramide
GCP	Good Clinical Practice
GCS	Glucosylceramide synthase
GI	Gastrointestinal
GLA	Gene coding for α-galactosidase A
GlcCer	Glucosylceramide (Gb1)

GSL	Glycosphingolipid
HbA1c	Hemoglobin A1c
HR	Heart rate
i.v.	Intravenous
IB	Investigator's Brochure
IBS	Irritable bowel syndrome
ICE	Intercurrent event
ICF	Informed consent form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMMPACT	Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials
IPMP	Individualized Pain Management Plan
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISF	Investigator Site File
J2R	Jump to Reference
K_i	Inhibition constant
λ_Z	Terminal disposition rate constant
LacCer	Lactosylceramide (Gb2)
LVEF	Left ventricular ejection fraction
LVMI	Left ventricular mass index
lysoGb3	Globotriaosylsphingosine
MAP	Mean arterial pressure
MAR	Missing at random
MedDRA™	Medical Dictionary for Regulatory Activities
mFAS	Modified Full Analysis Set
mFAS-GIS	Modified Full Analysis Set-gastrointestinal symptoms
MI	Multiple imputation
MMRM	Mixed model for repeated measures
MNAR	Missing not at random

NRS-11	11-point numerical rating scale
NSAID	Non-steroidal anti-inflammatory drug
NYHA	New York Heart Association
OCT2	Organic cation transporter 2
OLE	Open-label extension
ORc	Common odds ratio
PD	Pharmacodynamic(s)
PGIC	Patient Global Impression of Change
PGIC-DS	Patient Global Impression of Change in Disease Severity
PGIC-PS	Patient Global Impression of Change in neuropathic Pain Severity
PGIS-D	Patient Global Impression of Severity of Disease
PGIS-P	Patient Global Impression of Severity of neuropathic Pain
PI	Principal investigator
PK	Pharmacokinetic(s)
PPS	Per-Protocol Analysis Set
PRO	Patient Reported Outcome
PTOP	Post-treatment observation period
QoL	Quality of life
QS	Quality System
QTc	Corrected QT
QTcB	QT corrected according to Bazett's formula
QTcF	QT corrected according to Fridericia's formula
RSI	Reference safety information
SAE	Serious adverse event
SAF	Safety Set
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SD	Standard deviation
SF-36v2™	36-Item Short Form Health Survey Version 2
SIV	Site initiation visit
SNRI	Serotonin-norepinephrine re-uptake inhibitor
SOC	System organ class

SRT	Substrate reduction therapy
SSRI	Selective serotonin re-uptake inhibitor
SUSAR	Suspected unexpected serious adverse reaction
$t_{½}$	Apparent terminal elimination half-life
TCA	Tricyclic antidepressant
t_{max}	Time to reach maximum observed plasma concentration
TQT	Thorough QT
UACR	Urine albumin-to-creatinine ratio
ULN	Upper limit of normal
VHP	Voluntary harmonization procedure

NON-SUBSTANTIAL GLOBAL AMENDMENT 4

Rationale for the amendment

This applies to global protocol ID-069A301 Version 4 dated 1 October 2020. The resulting amended global protocol is Version 5 dated 24 August 2021.

In addition to this protocol and its amendments, a separate addendum to the protocol is in place to cover exceptional measures to ensure subject safety in the context of the COVID-19 pandemic and counteract any trial conduct disruption that may occur. The addendum applies to sites affected by the COVID-19 outbreak and is limited to the time during which such sites are affected.

The main purpose of this fourth global amendment is to change the statistical analysis of the secondary endpoint, Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale (BSS) consistency Type 6 or 7, from a parametric analysis (assuming normality) to a non-parametric analysis. This change in analysis is warranted because blinded baseline data review suggests that the endpoint is not normally distributed.

The necessity to make a change to this analysis method was discussed with the U.S. Food and Drug Administration (FDA); the change is restricted to the statistical analysis of the last secondary endpoint in the testing strategy and does not alter the study population and the primary and secondary objectives of the study.

Changes to the protocol

Two versions of the amended protocol will be prepared: 1) a clean version and 2) a Word comparison document showing deletions and insertions in comparison to the previous protocol version.

Amended protocol sections

The main sections of the protocol affected by this global amendment are listed below. Where applicable, the same changes have also been made to the corresponding sections of the protocol synopsis:

Section 6.1.3	Efficacy estimands
Section 10.3.2.1	Hypotheses
Section 10.3.3.2	Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale consistency Type 6 or 7 in subjects with gastrointestinal symptoms at baseline
Section 13	References

Summary of previous amendments:

Amendment	Date	Main reason(s)
1	12 April 2018	Non-substantial global amendment to address questions and comments received as part of the FDA review on 9 March 2018 (items 1 and 2) and the VHP assessments on 20 February 2018 (items 3 to 5) and 20 March 2018 (item 6): 1. The definition of neuropathic pain was split to improve readability and patient comprehension. The descriptor "numbness" was removed from the description as it was considered a distinct concept that does not necessarily describe neuropathic pain severity. The concept of intermittence was also added to the definition of neuropathic pain. 2. A new patient global impression of severity scale to assess the overall severity of neuropathic pain was added. 3. It was clarified that current guidelines for the diagnosis and treatment of Fabry disease were to be followed. 4. A more explicit definition of the global end of the study was included. 5. The process of the electronic diary (eDiary) data availability, transfer, encoding, and the measures taken to maintain privacy of the patient was clarified. 6. Exclusion criteria were adjusted to emphasize that any subject at high risk of developing clinical signs of organ involvement within the time period of the study, as per investigator judgment would be excluded from the study. In addition, the following modifications were made: • The Discussion Guide for the Exit Interview was removed from the appendices as it is only relevant for the substudy conducted in the US and Canada. It was revised based on FDA recommendations and was submitted separately in those two countries.

		• The time when the subject must definitively return the eDiary to the site personnel was clarified. • Reporting of change in intensity of an AE was clarified. • Footnote 'h' in Table 4 was corrected as it was not consistent with corresponding Section 7.2.4.2.1 of the protocol. • Footnote 'a' in Table 5 was removed as it was not consistent with Section 3.1.1, footnote 'a' of Table 4 and protocol synopsis. • A typographical error in Table 5 was corrected (serum sampling moved from biomarker sampling line to research sampling line) as it was not consistent with Sections 7.2.7.1 and 7.2.7.2 of the protocol. • The sequence of site visit assessments was completed. • The stool sample collection was further clarified. • The nonclinical summary section was updated to detail the toxicological findings.
2	25 October 2018	Substantial global amendment to remove some of the protocol restrictions following the release of the Investigator's Brochure version 8 (October 2018): • The results of a clinical drug-drug interaction (DDI) study (ID-069-105) with the organic cation transporter 2 (OCT2) inhibitor cimetidine led to the conclusion that lucerastat can be administered concomitantly with OCT2 inhibitors without need for dose adaptation. Therefore, respective protocol restrictions at study entry (exclusion criterion #15) and during the study (forbidden concomitant therapy) related to the use of OCT2 inhibitors have been removed. In addition, the following modifications were made:

		• The "Current treatment of Fabry disease" section has been updated to reflect the approval of Galafold® outside of Europe; • The clinical summary section has been updated to include the results of the clinical DDI study with cimetidine; • The conditions under which barrier contraception can be used and the list of methods of contraception that are not considered as acceptable have been updated in line with the '*Recommendations related to contraception and pregnancy testing in clinical trials'* version 15-09-2014 from the Clinical Trials Facilitation Group; • The sequence of the Patient Global Impression questionnaire assessments was corrected; • The stool sample collection and shipment process was updated in line with the central laboratory manual and study-specific laboratory flowchart.
3	1 October 2020	Global amendment to change the method used to analyze the primary endpoint using a dichotomous responder analysis, based on a reduction of at least 30% from baseline to Month 6 in modified Brief Pain Inventory-Short Form item 3 (BPI-SF3) score, to an analysis of the change from baseline to Month 6 in modified BPI-SF3 score. The dichotomous responder analysis will be documented to support and establish clinical relevance. The change in the analysis of the modified BPI-SF3 score was agreed with the FDA. It was restricted to the primary statistical analysis and did not alter the study population and the primary objective of the study to determine the effect of lucerastat on neuropathic pain in subject with Fabry disease. In addition, the following modifications were made, including: • Improvements to statistical methodology as a result of FDA requests and advice, including:

- Efficacy estimands targeted by the primary and secondary analyses have been added;
- A multiple testing procedure to control the study-wise two-sided type I error at 0.05 or less has been added. Consequently, the two secondary endpoints based on gastrointestinal symptoms are no longer tested with a two-sided significance level of 0.10;
- The number of subjects expected in the modified Full Analysis Set-gastrointestinal symptoms population has been clarified, including power estimates for a range of treatment effects;
- The use of baseline/last/worst observation carried forward imputation methods for the primary and secondary endpoints analyses have been replaced by multiple imputation methods;
- Multiple alternative definitions of the primary endpoint using different approaches of handling missing data have been removed;
- Following the change from a responder to a continuous primary analysis method, the analysis for assessing the impact of rescue pain therapy has been added. This now replaces the responder analyses to assess the impact of data collected during the post-treatment observation period and/or after initiation of enzyme replacement therapy and/or after changes or initiation of significant rescue pain therapy;
- As a responder analysis is no longer the primary analysis method, the related supportive analysis using logistic regression (also a responder analysis) has been removed;

- To fulfill the clinical commitment made to the Irish Health Products Regulatory Authority at the time of clinical trial approval, the investigational treatment description was updated to specify the amount of lactose intake by the subjects. In addition, the exclusion criteria (8b and 11) were updated to emphasize the exclusion of subjects with galactose intolerance, total lactase deficiency, glucose-galactose

	malabsorption, and subjects with hypersensitivity to lactose as lucerastat excipient; • The clinical summary section has been updated to include the results of the thorough QT study; • The exit interview section has been updated to incorporate FDA requirements; • The time windows for study assessments and study treatment dispensing have been revised to accommodate logistics at sites; • The recording of study treatment interruption in the electronic case report form has been further clarified; • The definition of re-tests and what is needed during unscheduled visits has been clarified.

PROTOCOL SYNOPSIS ID-069A301

TITLE	A multi-center, double-blind, randomized, placebo-controlled, parallel-group study to determine the efficacy and safety of lucerastat oral monotherapy in adult subjects with Fabry disease.
ACRONYM	MODIFY
OBJECTIVES	**Primary objective** The primary objective of the study is to determine the effect of lucerastat on neuropathic pain in subjects with Fabry disease (FD). **Secondary objectives** • To determine the effects of lucerastat on gastrointestinal (GI) symptoms (abdominal pain and diarrhea) in subjects with FD and GI symptom(s) at baseline; • To confirm the effect of lucerastat on biomarkers of FD; • To determine the safety and tolerability of lucerastat in subjects with FD. **Other objectives** Other objectives are described in Section 2.3 of the core protocol.
DESIGN	This is a prospective, multicenter, double-blind, randomized, placebo-controlled, parallel-group, Phase 3 study. Approximately 99 adult subjects with FD exhibiting Fabry-associated pain of moderate to severe intensity will be randomized in a 2:1 ratio to either lucerastat (approximately 66 subjects) or placebo (approximately 33 subjects). Treatment allocation will be stratified by sex and by specific background FD treatment at screening (subjects treated with enzyme replacement therapy [ERT], also called "switch" subjects, as they will have to stop ERT at screening visit, vs subjects not treated with ERT at screening).

	Subjects not treated with ERT at screening include: (i) "treatment-naïve" subjects who have never been treated with ERT. (ii) "pseudo-naïve" subjects who stopped ERT at least 6 months prior to screening. Once randomized, subjects will enter a 6-month double-blind treatment period. The study comprises the following consecutive periods: **Screening period:** Lasts approximately 6–7 weeks; starts with the signing of the informed consent form (ICF; at the screening visit) and ends the day before subject randomization. **Treatment period:** Lasts approximately 6 months; Starts on the day of subject randomization (randomization visit) and ends at the End-of-Treatment (EOT) visit (Month 6). **Post-treatment observation period (PTOP):** Subjects who discontinue study treatment prematurely will enter into the PTOP which starts on the day after the last dose of study treatment, and ends at latest at the Month 6 PTOP visit. **Post-treatment safety follow-up (FU) period:** The FU period is applicable to all subjects except those who enter the open-label extension (OLE) study. It starts on the day after the last dose of study treatment: • For female and non-fertile male subjects: it includes 1 safety FU telephone call (FU1) taking place approximately 1 month after the last dose of study treatment; • For fertile male subjects: it includes 2 safety FU telephone calls taking place approximately 1 month (FU1) and 3 months (FU2) after the last dose of study treatment. Subjects who complete the 6-month double-blind treatment period will be proposed to enroll into an OLE study conducted under a separate protocol (provided the extension study protocol has been approved in the country/site by regulatory

	authorities and Ethics Committees [ECs] / Institutional Review Boards [IRBs]). Subjects who discontinue study treatment prematurely for any reason should be subsequently treated according to local standard-of-care at the investigator's discretion and will be followed in the PTOP until the originally scheduled Month 6 visit.
PLANNED DURATION	Approximately 3.5 years from first subject, first visit to last subject, last visit. The global end of the study corresponds to the last visit of the last subject: • If the last subject enters the OLE study, the global end of the study corresponds to the last subject's EOT visit. • If the last subject does not enter the OLE study, the global end of the study corresponds to the last subject's FU1 visit, FU2 visit, or to the last visit of the PTOP, whichever is last.
SITES/COUNTRIES	Approximately 58 sites in approximately 14 countries.
INCLUSION CRITERIA	**Screening visit criteria** 1. Signed and dated ICF prior to any study-mandated procedure; 2. Male or female subjects 18 years old and above; 3. FD diagnosis confirmed with local genetic test results (i.e., presence of at least 1 mutation in *GLA*, the gene coding for α-galactosidase A [α-GalA]); 4. Fabry-associated neuropathic pain, as defined by the subject, in the last 3 months prior to screening; 5. ERT treatment status: a) Subject never treated with ERT; or b) Subject has not received ERT for at least 6 months prior to screening; or

	c) Subject treated with ERT at the time of the screening visit and meeting all of the following criteria at the time of screening: i) ERT administration for the last 12 months; ii) Stable ERT dose regimen during the last 3 months; iii) Subject agrees to stop ERT administration at the screening visit for approximately 8 months (6–7 weeks screening + 6 months of double-blind treatment). 6. A woman of childbearing potential [see definition in Section 4.5.1 of the core protocol] is eligible only if the following applies: – Negative serum pregnancy test at screening and a negative urine pregnancy test at randomization; – Agreement to undertake monthly urine pregnancy tests during the study and up to at least 30 days after study treatment discontinuation; – Agreement to follow a highly effective contraception scheme as described in Section 4.5.2 of the core protocol from screening up to at least 30 days after study treatment discontinuation. 7. A fertile male (physiologically capable of conceiving a child according to investigator judgment) who is sexually active with a woman of childbearing potential is eligible only if the following applies: – Agreement to use a condom during the treatment period (starting at randomization) and for up to 3 months after study treatment discontinuation; and – Agreement not to father a child during this period. **Randomization visit criteria** 8. Adequate subject compliance with completion of an eDiary during the screening period; 9. Subjects with moderate or severe neuropathic pain as determined from daily entries of the modified Brief Pain Inventory-Short Form item 3 (BPI-SF3) score of

	"neuropathic pain at its worst in the last 24 hours" in the eDiary during the screening period. The definition of moderate to severe neuropathic pain at baseline is provided in Section 4.3 of the core protocol.
EXCLUSION CRITERIA	**Screening visit criteria** ***Disease/condition*** 1. Pregnant / planning to become pregnant up to 30 days after study treatment discontinuation or lactating subject; 2. Severe renal insufficiency defined as an estimated glomerular filtration rate (eGFR) per the Chronic Kidney Disease Epidemiology Collaboration creatinine equation < 30 mL/min/1.73 m^2 at screening (as reported by the central laboratory); 3. Subject on regular dialysis for the treatment of chronic kidney disease; 4. Subject has undergone, or is on a waiting list for, or is scheduled to undergo kidney or other organ transplantation; 5. Known and documented transient ischemic attack, stroke, unstable angina or myocardial infarction within 6 months prior to screening; 6. Clinically significant unstable cardiac disease in the opinion of the investigator (e.g., uncontrolled symptomatic arrhythmia, New York Heart Association class III or IV congestive heart failure); 7. Any other subject at high risk of developing clinical signs of organ involvement within the time period of the study, as per investigator judgment; 8. Any known factor or disease that might interfere with treatment compliance, study conduct or interpretation of the results such as: a) Other disease or condition associated with a pain component that could confound assessment of neuropathic pain (e.g., diabetic neuropathy, chemotherapy- or radiation-induced peripheral

	neuropathy, chronic inflammatory demyelinating polyneuropathy); b) Other disease of the GI tract that could interfere with the assessment of GI symptoms in FD (e.g., inflammatory bowel disease, galactose intolerance, total lactase deficiency or glucose-galactose malabsorption); c) Documented poorly controlled diabetes mellitus (i.e., HbA1c > 8.0% at screening as reported by the central laboratory); d) Significant neurological disorder; e) Significant psychiatric disease; suicidal ideation at screening or history of suicide attempt or behavior within 6 months prior to screening as per investigator judgment; f) History of drug dependence (including opioids) or alcohol dependence; g) Inability to complete an eDiary on a daily basis. 9. Known concomitant life-threatening disease with a life expectancy < 18 months. ***Treatments*** 10. Subject planned for imminent initiation of treatment with ERT; 11. Known hypersensitivity to lucerastat or drug of the same chemical class of iminosugars (e.g., miglitol, miglustat, migalastat), or any of their excipients (including lactose); 12. Initiation or treatment at an unstable dose within 4 weeks prior to screening with any of the following medications: a) Angiotensin-converting enzyme (ACE) inhibitor and/or angiotensin receptor blocker (ARB); b) Anti-epileptic; c) Tricyclic antidepressant (TCA) and/or other antidepressants belonging to the serotonin-norepinephrine re-uptake inhibitor (SNRI)

	and selective serotonin re-uptake inhibitor (SSRI) classes. 13. Planned or current treatment with another investigational treatment within 3 months prior to screening; 14. Treatment with any inhibitor of the glucosylceramide synthase (GCS) (e.g., miglustat, lucerastat, eliglustat, ibiglustat/venglustat) or an α-GalA chaperone (e.g., migalastat) within 6 months prior to screening; **Randomization visit criteria** 15. Treatment with ERT (agalsidase alfa, agalsidase beta) during the screening period.
STUDY TREATMENTS	Lucerastat is currently available for clinical study use as hard gelatin capsules containing 250 mg of lucerastat and inactive excipients ([redacted] mg of lactose anhydrous and [redacted] mg of talc). Placebo capsules will be identical in appearance to the lucerastat capsules, and will contain inactive excipients ([redacted] mg of lactose anhydrous and [redacted] mg of talc). The starting dose of the study treatment (lucerastat or matching placebo) will be based on the subject's eGFR value (as reported by the central laboratory) at the screening visit as shown in Table 1 of the core protocol. During the study, the dose of the study treatment will be reduced if the subject's eGFR (as reported by the central laboratory during scheduled or unscheduled visits) decreases and crosses the next lower eGFR boundary as shown in Table 1 of the core protocol. Study treatment must be discontinued if one of the study-treatment stopping criteria is met [see Section 5.1.10 of the core protocol].
CONCOMITANT THERAPY	**Mandatory concomitant therapy** Mandatory therapy includes any treatments required for contraception purposes in women of childbearing potential.

	Allowed concomitant therapy Treatments considered necessary for the subject's well-being and not categorized as forbidden concomitant medications are allowed during the study and must be documented in the medical charts. ***ACE inhibitor or ARB*** Subject must not have initiated ACE inhibitor or ARB therapy within 4 weeks prior to screening. ACE inhibitor or ARB therapy, if used by the subject, must be at a stable dose for at least 4 weeks prior to screening. During the study, initiation or dose adjustment is allowed based on investigator judgment. ***Pain medications*** During the study, the use of adjuvant pain medications, non-opioid and opioid analgesics is allowed if judged medically required by the investigator. Subject must not have initiated adjuvant pain medications (i.e., anti-epileptics, TCAs and SNRIs/SSRIs) within 4 weeks prior to screening. The dose regimen of adjuvant pain medications used chronically by the subject at screening must be stable for at least 4 weeks prior to screening. In the event of unbearable pain, rescue pain medications may be considered. An Individualized Pain Management Plan (IPMP) will be provided by the investigator/delegate to each subject with customized instructions regarding the use of pain medications during the study. The IPMP will list the pain medications ongoing at the screening visit that the subject should continue taking during the study. It will also include a list of new pain medication(s) or dose adjustment that should be made in the event of unbearable pain taking into account the subject's background pain medication(s) and previous response to pain medication(s). The IPMP will be applicable starting at screening visit and until the EOT visit or the last visit of the PTOP (as applicable). Depending on the background pain medication(s) of the subject, and provided that the medication is not categorized as forbidden concomitant medication [Section 5.2.4], the rescue

	pain medication(s) may include one or more of the following options: • Initiation or dose escalation of adjuvant pain medication(s) (i.e., anti-epileptics, TCAs, SNRIs, SSRIs); • Initiation or dose escalation of opioid analgesic drugs; • Initiation or dose escalation of non-opioid analgesics drugs (i.e., non-steroidal anti-inflammatory drug, topical analgesics). If, after the modification of pain treatment, the subject still suffers from unbearable pain, the investigator/delegate should discuss with the subject alternative therapeutic options including the possibility to (re-)initiate ERT treatment (if available at the site). ***Antidepressants used to treat depression*** Subject must not have initiated antidepressants (i.e., SNRIs, SSRIs, TCAs) within 4 weeks prior to screening. The doses of antidepressants used by the subject at screening must be stable for at least 4 weeks prior to screening. During the study, initiation or dose adjustment are allowed based on investigator judgment. **Forbidden concomitant therapy** ***ERT (agalsidase alfa, agalsidase beta)*** Subjects on ERT with neuropathic pain at screening ("switch") must agree to stop ERT administration for about 8 months (6–7 weeks screening + 6 months of double-blind treatment). Recently published recommendations by the European Fabry Working Group have defined ERT stopping criteria which include the lack of response for 12 months when the sole "indication" for ERT is neuropathic pain, and the subject's request to stop ERT [Biegstraaten 2015]. Subjects off ERT with neuropathic pain at screening ("naïve" or "pseudo-naïve") who are planned for imminent initiation of treatment with ERT are not eligible for the study [see Section 4.4 of the core protocol], and initiation of ERT will therefore not be withheld for these subjects.

	Although discouraged throughout the whole study, subjects may be allowed to (re-)initiate ERT treatment (if available at that site) if judged medically required due to significant FD progression (e.g., significant renal function deterioration or cardiac function deterioration) based on investigator judgment. ***Inhibitors of GCS, α-GalA chaperone, investigational drugs*** The following concomitant therapies are forbidden from screening visit until the EOT visit or the last visit of the PTOP (as applicable): • Any inhibitor of GCS (e.g., miglustat, eliglustat); • Any α-GalA chaperone (e.g., migalastat); • Any other investigational drug (e.g., ibiglustat/venglustat, pegunigalsidase alfa).
ENDPOINTS	**Primary efficacy endpoint** The primary efficacy endpoint is change from baseline to Month 6 in the "modified" BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours". **Secondary efficacy endpoints** • Change from baseline to Month 6 in the 11-point numerical rating scale (NRS-11) score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline. The definition of a subject considered to have GI symptoms at baseline is provided in Section 10.1.4 of the core protocol. • Change from baseline to Month 6 in the number of days with at least one stool of a Bristol Stool Scale (BSS) consistency Type 6 or 7 in subjects with GI symptoms at baseline; • Change from baseline to Month 6 in plasma globotriaosylceramide (Gb3). **Other efficacy endpoints** Other efficacy endpoints are described in Section 6.1.4 of the core protocol.

	Safety endpoints • Treatment-emergent adverse events (AEs) and serious AEs; • AEs leading to premature discontinuation of study treatment; • Change from baseline to each visit up to Month 6 in vital signs; • Treatment-emergent marked abnormalities for vital signs variables up to Month 6; • Change from baseline to each visit up to Month 6 and from pre-dose to 2 hours and 4 hours post dose at Month 1 in 12-lead electrocardiogram (ECG) variables; • Treatment-emergent marked abnormalities for quantitative 12-lead ECG variables up to Month 6; • Change from baseline to each visit up to Month 6 in laboratory variables [see list in Section 7.2.4.2 of the core protocol]; • Treatment-emergent marked abnormalities for selected laboratory variables up to Month 6. **Other endpoints (quality of life, pharmacokinetic [PK], other biomarkers)** Other endpoints are described in Sections 6.3, 6.4, and 6.5 of the core protocol.
ASSESSMENTS	Refer to the schedule of assessments in Table 5 and Table 6 of the core protocol.
STATISTICAL METHODOLOGY	**Analysis sets** The Screened Analysis Set includes all subjects who have given informed consent to participate in the study and have a subject number. The Full Analysis Set (FAS) includes all subjects randomized to either lucerastat or placebo. Subjects will be evaluated according to their assigned study treatment and stratum information as recorded in the Interactive Response Technology system.

	A modified FAS (mFAS) will be defined by including subjects from the FAS who took at least 1 dose of study treatment. The Per-Protocol Analysis Set includes all subjects from the mFAS without important protocol deviations occurring prior to Month 6, which could affect the analysis of the primary endpoint variable. The mFAS-gastrointestinal symptoms (mFAS-GIS) comprises all subjects from the mFAS who, during the 4 weeks prior to randomization, have experienced: • abdominal pain of moderate to severe intensity at baseline defined as an average abdominal pain intensity score ≥ 3 on an NRS-11 scale; and/or • diarrhea at baseline defined as having at least 1 stool of a BSS consistency type 6 or 7 on at least 8 days. The Safety Set (SAF) includes all subjects who received at least 1 dose of study treatment (as recorded in the electronic case report form). Subjects will be analyzed based on the treatment received. In the event of accidental dispensation of both study treatments to the same subject, the subject will be counted as on lucerastat for the whole study. Other analysis datasets will be defined in the statistical analysis plan (SAP; or corresponding SAPs), e.g., PK set, sub-study sets, and subgroups of interest. **Overall testing strategy** Comparisons of lucerastat vs placebo will be conducted for the primary and secondary endpoints assessed at Month 6. The Type I error rate will be controlled at a two-sided alpha of 5% for the testing of the four null hypotheses associated with the primary and secondary endpoint comparisons employing a fixed-sequence statistical testing strategy in the following order: 1. Change from baseline to Month 6 in the “modified” BPI-SF3 score of “neuropathic pain at its worst in the last 24 hours”.

	2. Change from baseline to Month 6 in plasma Gb3. 3. Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline. 4. Change from baseline to Month 6 in the number of days with at least one stool of a BSS consistency Type 6 or 7 in subjects with GI symptoms at baseline. All available data will be used regardless of occurrence of intercurrent events such as premature treatment discontinuation or changes in background medication. **Analysis of the primary efficacy variable** Hypotheses for the primary endpoint are formulated in terms of the mean differences in change from baseline to Month 6. H_0: Lucerastat - Placebo = 0 is the null hypothesis that there is no difference between treatments. H_A: Lucerastat - Placebo ≠ 0 is the alternative hypothesis that a difference in change from baseline to Month 6 exists between treatments. The primary statistical analysis will be performed on the mFAS, according to the intent-to-treat approach. The null hypothesis will be tested at the two-sided alpha level = 0.05, using the following method: Missing data will be imputed applying a control-based multiple imputation assuming missing not at random using the Copy Reference approach [Carpenter 2013]. Instead of imputing a single value for each missing observation, a set of values is generated from the model, resulting in as many distinct complete datasets without missing data. The imputation model includes the baseline value, the two stratification factors (sex and ERT treatment status at screening) and all post-baseline monthly scores up to Month 6. Missing data for subjects from both treatment arms will be imputed using data from the placebo arm. This approach assumes that subjects with missing

	data in the lucerastat arm have outcomes trending towards outcomes observed in the placebo arm, i.e., the imputations result in a treatment effect that gradually diminishes towards the placebo arm. An analysis of covariance model will be used to analyze this endpoint on each imputed dataset. The following terms will be included in the model: baseline value, the two stratification factors (sex and ERT treatment status at screening), and the treatment group. Uncertainty in the imputations will be reflected appropriately in the analysis by combining the results on each imputed dataset using Rubin's methodology [Rubin 1987]. The mean difference in changes from baseline to Month 6 between lucerastat and placebo together with its two-sided 95% confidence interval and p-value will be reported. The robustness of inferences from the primary endpoint analysis to deviations from its underlying modelling assumptions will be explored using sensitivity analyses. A series of additional analyses based on the modified BPI-SF3 score data will support the primary endpoint results by analyzing the primary endpoint variable using various other methods and assumptions. Sub-group analyses classifying subjects according to important baseline characteristics, will be performed to explore the consistency of treatment effect in a variety of relevant subgroups to support the efficacy evaluation of lucerastat in this indication. **Analysis of the secondary efficacy variables** The following secondary efficacy endpoint variables will be analyzed in the mFAS-GIS at a two-sided significance level of alpha = 0.05: • Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline;

	• Change from baseline to Month 6 in the number of days with at least 1 stool of a BSS consistency Type 6 or 7 in subjects with GI symptoms at baseline. The following secondary efficacy endpoint variable: • Change from baseline to Month 6 in plasma Gb3 will be analyzed in the mFAS at the two-sided significance level of alpha = 0.05. **Sample size** The planned sample size for this study is 99 subjects using a 2:1 allocation ratio (66 lucerastat, 33 placebo) and is primarily based on a test for the mean difference between lucerastat and placebo in the change from baseline to Month 6 in the "modified" BPI-SF3 score. Assuming a clinically relevant difference of 2 points on the 11-point scale (0–10) between lucerastat and placebo, a corresponding standard deviation of 3 points, a two-sided type I error of 5%, and equal group variances, 99 subjects will provide a power of 87.2% to detect a treatment difference between lucerastat and placebo on the primary endpoint. The calculations were conducted using East 6.5 based on a two-sided t-test for independent samples.
STUDY COMMITTEE	An Independent Data Monitoring Committee (IDMC) will have the overall responsibility for safeguarding the interests of subjects by monitoring safety and efficacy data obtained in the study and making appropriate recommendations based on the reported data, thus ensuring that the study is being conducted to the highest scientific and ethical standards. The IDMC will be fully operational prior to enrollment of the first subject into the study. The composition and operation of the IDMC will be described in an IDMC charter.
SUB-STUDIES	**PK profile sub-study (all sites)** A sub-study will be conducted at all sites to determine the PK profile of lucerastat at steady state in a subgroup of approximately 36 subjects with FD with either normal or subnormal renal function (i.e., screening

	eGFR ≥ 60 mL/min/1.73 m^2) or impaired renal function (i.e., screening eGFR < 60 mL/min/1.73 m^2). If necessary, blood samples collected for PK may be used for the identification of potential metabolites of lucerastat in this population. Subjects consenting to this sub-study will have a 12-hour PK profile performed at the Month 1 visit. **Exit interview sub-study (US and Canadian sites only)** An exit interview sub-study will be conducted in all English-speaking subjects at US and Canadian sites. The interviews will focus on gaining an understanding of what constitutes a meaningful change on the neuropathic pain and GI symptom (diarrhea and abdominal pain) questions from a subject perspective. Details on the analysis of the exit interview data are available in the Exit Interview Analysis Plan.
STUDY EXTENSION	Subjects who complete the 6-month double-blind treatment period will be proposed to enroll into an OLE study conducted under a separate protocol (provided the OLE study protocol has been approved in the country/site by regulatory authorities and ECs/IRBs at the time of enrollment).

PROTOCOL

1 BACKGROUND

1.1 Fabry disease

Fabry disease (FD) is a rare, pan-ethnic X-linked inherited glycosphingolipid (GSL) storage disorder caused by the deficiency of the lysosomal enzyme α-galactosidase A (α-GalA) [Germain 2010]. In FD, mutations in the gene coding for α-GalA (*GLA* gene), located on the X chromosome, are responsible for reduced expression and/or activity of α-GalA resulting in accumulation of globotriaosylceramide (Gb3), globotriaosylsphingosine (lysoGb3), and other neutral GSLs in lysosomes and other subcellular compartments. Progressive storage of these GSL molecules eventually leads to cellular dysfunction, associated with inflammation and/or fibrosis which results in multisystem disease, mainly affecting the kidneys, heart, and nervous system.

Diagnosis of FD combines clinical presentation, α-GalA activity and *GLA* mutations, as defined in the most recent international guidelines [Schiffmann 2017].

There are 2 major phenotypes: a classic phenotype and a late-onset (also referred to as "atypical" or "non-classical") phenotype [Arends 2017].

The classic phenotype is more severe due to little or no α-GalA activity. The first clinical symptoms (e.g., neuropathic pain, gastrointestinal [GI] symptoms, abnormal sweating) interfering with well-being and daily activities arise in childhood [Hopkin 2008]. In the absence of treatment, progressive damage to vital organ systems occurs in subjects with FD over several decades, resulting in end-stage renal disease and/or life-threatening cardiovascular or cerebrovascular complications, causing substantial morbidity, significantly impaired quality of life (QoL), and premature death [MacDermot 2001a, MacDermot 2001b].

The late-onset phenotype is typically less severe and is observed in subjects with significant residual α-GalA activity. It is characterized by a more variable disease course, and disease manifestations may be limited to a single organ, e.g., the heart.

As expected for an X-linked disorder, males with deleterious mutations have little or no residual α-GalA activity. Therefore, these male subjects usually experience the full spectrum of disease symptoms. In heterozygous female subjects with FD (carriers) who have a mutation in one copy of the *GLA* gene, random X inactivation effectively results in mosaic expression, with some cells exhibiting normal α-GalA activity and others with little or no α-GalA activity [Linhart 2007]. The severity of the disease is heterogeneous in female carriers, who, like male subjects and somehow unexpectedly, may develop life-threatening complications of the disorder. Up to 70% of female carriers develop FD-related symptoms, including neuropathic pain, GI symptoms, renal and cardiac disease,

and/or stroke [Schiffmann 2009]. In general, affected female carriers experience symptoms similar to hemizygous males with a delayed onset and slower progression compared to men [Linhart 2007].

1.1.1 Current treatment of Fabry disease

The management of FD consists of conventional palliative management, enzyme replacement therapy (ERT), and chaperone therapy.

Conventional management consists of pain medications, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) to protect the heart and kidney, antiarrhythmic agents, and anticoagulants to avoid thrombotic complications. Dialysis or renal transplantation are available for subjects experiencing end-stage renal failure. A recent review of the pharmacotherapy for neuropathic pain in Fabry adult subjects recommends using anti-epileptics (e.g., carbamazepine, gabapentin), tricyclic antidepressants (TCAs; e.g., amitriptyline), and other antidepressants of the serotonin-norepinephrine re-uptake inhibitor class (SNRIs; e.g., duloxetine, venlafaxine) as first-line therapy [Finnerup 2015, Politei 2016]. Non-opioid and opioid analgesic drugs are used as second- and third-line treatments, respectively. Due to the limited evidence with respect to the effectiveness of pain medication in FD, clinicians usually rely on local clinical experience or follow national and international guidelines for the management of neuropathic pain [Finnerup 2015].

Two injectable ERTs have been granted market authorization in at least 1 country for the treatment of FD: agalsidase alfa (Replagal®) and agalsidase beta (Fabrazyme®). However, their effectiveness in controlling all symptoms and complications of FD is limited (see below). Chaperone therapy is also available to improve the activity of some defective enzymes. In FD, migalastat (Galafold®), an oral small molecule pharmacological chaperone, has been granted market authorization in at least 1 country for the treatment of subjects with amenable mutations.

The most recent international guidelines provide the framework for screening, diagnosis and management of patients with FD [Biegstraaten 2015, Schiffmann 2017]. They include recommendations on the use of ERT, the discontinuation of ERT, the monitoring of Fabry-specific symptoms and organ diseases, and the use of adjunctive therapy. Recommendations for diagnosis and management of neuropathic pain in subjects with FD are also available [Politei 2016].

1.1.2 Unmet medical need in Fabry disease

Despite ERT administration, a large proportion of subjects with FD still exhibit clinical symptoms, in particular neuropathic pain, which impact their daily life. Although an effect on neuropathic pain after 6 months of treatment with agalsidase alfa has been suggested in a placebo-controlled study [Schiffmann 2001], the totality of the data provided no clear

evidence that ERT treatment is associated with improvement in the "worst pain" Brief Pain Inventory (BPI) scores. While no other placebo-controlled study was performed to assess the effect of ERT on neuropathic pain, further data from the Fabry Outcome Survey (FOS) registry [Hoffmann 2005] and a comparative trial with agalsidase alfa or beta [Vedder 2007] showed little or no improvement in neuropathic pain with ERT in subjects with FD.

The effects of ERT on different organs/systems in subjects with FD have been extensively evaluated [Eng 2001, Banikazemi 2007, Schiffmann 2001]. Overall, some limited improvements in renal function, cardiac mass, cerebrovascular complications, and QoL have been reported [Mehta 2009]. Long-term effects of ERT on risk of morbidity and mortality related to FD remain to be established even to this day [El Dib 2016].

ERT for FD has intrinsic limitations such as a very short half-life combined with a low tissue penetration, in particular in the nervous system [Murray 2007, Thurberg 2004, Thurberg 2009], and the development of antibodies to the infused protein in many subjects [Deegan 2012]. Inhibitory antibodies may compromise the efficacy of the treatment with ERT [Lenders 2016]. Furthermore, the intravenous (i.v.) route of administration for ERT is associated with an elevated risk of adverse events (AEs) and it can be inconvenient [Mehta 2009].

The oral administration of migalastat is restricted to subjects with amenable mutations only, which represent approximately 30% of all known *GLA* mutations [Galafold® SmPC]. In that population, the Phase 3 study results suggest that migalastat may provide some beneficial clinical effects, e.g., stabilizing renal function and decreasing left ventricular mass index (LVMI) [Germain 2016]. However, long-term data with the use of chaperone therapy in FD is tenuous.

Overall, there is still an unmet medical need in the treatment of subjects with FD. The available therapies have not yet demonstrated long-term clinical benefit on the main symptoms such as neuropathic pain. In addition, their use is either restricted due to their mode of administration (i.v. infusion) and immunogenic potential or indicated only in a subset of subjects with FD.

1.2 Lucerastat

1.2.1 Mechanism of action

Lucerastat is an iminosugar that has the potential to provide substrate reduction therapy (SRT) for the treatment of FD. The goal of SRT with lucerastat in FD is to inhibit the enzyme glucosylceramide synthase (GCS) that catalyzes the first committed step of GSL biosynthesis, thereby reducing the rate of synthesis of Gb3 to a level compatible with its residual clearance. The expected result is a reduction of net Gb3 load in tissues resulting in symptomatic improvement and a delayed progression towards end-stage organ failure.

1.2.2 Nonclinical summary

Nonclinical pharmacology studies were performed in mice. Pharmacokinetic (PK), metabolism, and toxicology *in vitro* and *in vivo* studies were performed in mice, rats, rabbits and dogs.

In summary, lucerastat dose dependently inhibits GCS at low micromolar concentrations, and reduces Gb3 storage and lysosomal staining in cultured FD subject fibroblasts, including fibroblasts from subjects with mutations associated with very low or no residual α-GalA activity [Welford 2017, Welford 2018]. In a mouse model of FD devoid of α-GalA activity oral lucerastat reduces Gb3 levels in the dorsal root ganglia (DRG) and in the kidney. In other mouse models of related GSL storage disorders such as Sandhoff disease, and G_{M1} gangliosidosis, lucerastat prolongs life span and improves clinical manifestations.

In rats and dogs, hepatic elimination is negligible and the main route of elimination is renal excretion of unchanged lucerastat. *In vitro* studies showed that lucerastat is a substrate of organic cation transporter 2 (OCT2), but not of other kidney transporters. This observation triggered a clinical drug-drug interaction (DDI) study with the OCT2 inhibitor cimetidine in healthy male subjects [see Section 1.2.3].

Dermal findings of low severity were present in rats and dogs. They consisted of acanthosis (epidermal hyperplasia), along with hyperkeratosis and lymphoid cell infiltration, in rats at doses ≥ 1000 mg/kg/day given for 26 weeks and in dogs at doses ≥ 300 mg/kg/day given for up to 39 weeks. The changes were mild, reversible, without further consequences, and thus considered not adverse. As skin findings were observed both in rodents and in non-rodents, and these findings might be related to exaggerated pharmacodynamic (PD) effects, it is recommended that the skin be monitored in clinical studies.

Embryo-fetal toxicity studies indicated that lucerastat has embryotoxic potential in rats and rabbits and teratogenic potential in rabbits at high doses inducing maternal toxicity.

In rat fertility studies, lucerastat had no effects on female fertility, while male fertility was reduced at doses ≥ 500 mg/kg/day. The 26-week toxicity study in rats showed changes in sperm parameters and microscopic findings in the testes and epididymides. In CD-1 mice and dogs, no changes in sperm parameters (assessed in the 13-week mouse study and the 4-week dog study) were seen. In addition, no histopathological effects in the testes were seen when lucerastat was administered for up to 13 weeks in mice and 39 weeks in dogs. Systemic exposure levels reached in mice and dogs were similar to or higher than those producing effects on the male reproductive system of rats. In the absence of changes in sperm parameters and microscopic findings in dogs and mice, the testicular toxicity observed in rats is considered unlikely to be relevant in man.

More detailed information can be found in the Investigator's Brochure (IB) [Lucerastat IB].

1.2.3 Clinical summary

A total of six Phase 1 studies have been completed, which overall enrolled 180 subjects: one single-ascending dose study (n = 39) and one multiple-ascending dose study (n = 37) in healthy male subjects, one study in subjects with different degrees of renal function impairment (n = 32), one exploratory Phase 1b study in subjects with FD (n = 14), one DDI study in healthy male subjects (n = 14), and one thorough QT (TQT) study in healthy subjects (n = 44). In the DDI study, lucerastat was administered concomitantly with the OCT2 inhibitor cimetidine at steady state.

Across the six Phase 1 studies, lucerastat has been administered to 152 subjects including 10 subjects with FD. It was found to be safe and well tolerated at all dose regimes evaluated (single oral doses up to 4000 mg, repeated oral doses up to 1000 mg twice daily [b.i.d.] for 12 weeks [on top of ERT], and a single oral dose of 500 mg administered concomitantly with cimetidine at steady state).

Overall, in the completed studies, there were 2 serious AEs (SAEs): One SAE of recurrent femoral thrombosis was reported in a subject with severe renal function impairment, and medical history of factor V Leiden gene mutation. Another SAE of re-occurrence of atrial fibrillation in the context of hypertrophic cardiomyopathy was reported in a subject with FD with a history of transient ischemic attack and atrial fibrillation.

Among other AEs, there was one with alanine aminotransferase (ALT) 5 × upper limit of normal (ULN) and aspartate aminotransferase (AST) 2.5 × ULN in a healthy subject leading to study drug discontinuation and full resolution thereafter. Total bilirubin remained within normal range. Few subjects in both lucerastat and placebo groups reported medically irrelevant AEs of increased liver enzymes.

After single dosing of lucerastat (range: 100–4000 mg) in healthy subjects, peak plasma drug concentrations were attained between 0.5 and 4.0 h, and the apparent terminal elimination half-life ($t_{½}$) ranged from 3.6–16.9 h. Following repeated administration of lucerastat (range: 200–1000 mg b.i.d.), peak plasma concentration observed during the dosing interval were attained between 0.5 h and 4.0 h. The PK of lucerastat were proportional for the area under the plasma concentration-time curve (AUC) extrapolated to infinity ($AUC_{0-\infty}$) over the dose range tested after both single and multiple dosing. There was no relevant increase in exposure from Day 1 to Day 7, indicating absence of accumulation over time. The time to reach steady state was approximately 2 days.

Renal clearance of unchanged lucerastat varied from 68–85% of oral clearance and mean renal excretion of unchanged lucerastat per daily dose from 68−85%. No clinically relevant food effect was observed.

The PK results of 1000 mg b.i.d. obtained in subjects with FD were in line with those observed in healthy subjects. The PK profiles of lucerastat were similar in subjects with

mild renal function impairment when compared with healthy subjects. Plasma lucerastat concentrations were higher in subjects with moderate and severe renal function impairment. The $t_{½}$ was longer in subjects with moderate (9.6 h) or severe renal function impairment (16.1 h) than in healthy subjects (7.0 h). Overall, this resulted in a 1.6- and 3.2-fold increase in $AUC_{0-\infty}$ of lucerastat in subjects with moderate and severe renal function impairment, respectively, when compared with healthy subjects.

In the DDI study with the OCT2 inhibitor cimetidine, 14 healthy male subjects received a single oral dose of 500 mg lucerastat (on Day 1), as well as a single oral dose of 500 mg lucerastat (on Day 6) administered concomitantly with cimetidine at steady state (multiple oral doses of 800 mg b.i.d. cimetidine from Day 3 to Day 9). Results from the DDI study demonstrated that cimetidine had only a weak effect on the exposure to lucerastat, i.e., the geometric mean (90% confidence interval [CI]) $AUC_{0-\infty}$ increased by 22% (16%; 27%) when lucerastat was administered concomitantly with cimetidine at steady state, as compared to lucerastat alone. Geometric mean (90% CI) maximum observed plasma concentration (C_{max}) was almost unchanged (i.e., increased by 4% [8%; 17%]) and absorption was slower (i.e., time to reach C_{max} [t_{max}] delayed by 1 h). Geometric mean $t_{½}$ was not changed. These effects are considered as not clinically relevant. Based on this, lucerastat can be administered concomitantly with OCT2 inhibitors without need for dose adaptation.

The TQT study was split into two parts: Part A was a double-blind, placebo-controlled study in healthy male subjects to determine the supratherapeutic dose of lucerastat to be used in Part B. Part B was a randomized, double-blind (for lucerastat), placebo-controlled, four-way crossover study including an open-label positive control in healthy male and female subjects to assess the effect of single therapeutic and supratherapeutic doses of lucerastat on the QT/QTc interval duration. In Part A, 6 subjects received a single oral dose of 2000 mg lucerastat on Day 1 and a single oral dose of 4000 mg lucerastat on Day 3, whereas 2 subjects received placebo both on Days 1 and 3. In Part B, 36 subjects were enrolled. Subjects received, in a random sequence, single oral doses of 1000 mg lucerastat (anticipated therapeutic dose), 4000 mg lucerastat (supratherapeutic dose), 400 mg open-label moxifloxacin (positive control to assess assay sensitivity), and placebo. Based on concentration-QTc analysis, a QTc according to Fridericia's formula (QTcF) effect of clinical concern (i.e., above 10 ms) could be excluded up to lucerastat plasma concentrations of approximately 34 µg/mL. The results constituted a negative TQT study, demonstrating that lucerastat up to a single dose of 4000 mg does not have any clinically relevant liability to prolong the QT interval or any clinically relevant effect on other ECG parameters.

In the exploratory Phase 1b study, a marked change in biomarkers was observed in subjects with FD who received 1000 mg b.i.d. lucerastat on top of ERT, with a mean percentage change from baseline (standard deviation [SD]) of –49% (17), –33% (13), and –55% (10)

in plasma levels of glucosylceramide (GlcCer), lactosylceramide (LacCer), and Gb3, respectively, at Week 12.

Urinary Gb3 was also decreased by 53% (21) at Week 12.

There was no change in plasma GlcCer, LacCer or Gb3 in the control group. No significant changes were observed for plasma lysoGb3 in either group. The concomitant decrease in GlcCer, LacCer, and Gb3 confirms GCS inhibition and α-GalA substrate reduction with lucerastat in subjects with FD.

More detailed information can be found in the IB [Lucerastat IB].

1.2.4 Lucerastat dose rationale

Based on the nonclinical data from studies in wild-type mice and Fabry mice [Lucerastat IB], it was hypothesized that achieving plasma drug concentrations equal to or above the inhibition constant (K_i) for inhibition of human GCS for at least 80% of the time during a 24 h dosing interval is needed to achieve relevant substrate reduction and subsequent decreases of plasma Gb3 levels in subjects with FD. The exploratory Phase 1b study in subjects with FD receiving ERT confirmed that treatment with a dose of lucerastat 1000 mg b.i.d. for 12 weeks was adequate, resulting in (i) plasma lucerastat concentration above the K_i for 98% of the time of a dosing interval, (ii) marked and favorable PD effects (e.g., a decrease in plasma Gb3 level of 55%), and (iii) good safety and tolerability [Lucerastat IB].

The nonclinical and clinical safety data and the resulting safety margins / exposure ratios are considered to adequately support the safe use of lucerastat at doses up to 1000 mg b.i.d. in long-term clinical studies in subjects with FD, taking into account the limited and/or species-specific toxicity in animals, the reversibility of the findings, and the severity of the disease.

Based on available nonclinical and clinical data, a dosing regimen of lucerastat 1000 mg b.i.d. is considered adequate to potentially achieve a clinical benefit while maintaining long-term safety and tolerability in subjects with FD.

In subjects with moderate renal function impairment increased systemic exposure was observed, hence dose adjustment is required in this population [see Section 5.1.2].

1.3 Rationale of the study

The rationale for this Phase 3 study is based on (i) the recognition of an unmet medical need of subjects with FD, (ii) the use of SRT with lucerastat as a distinct oral treatment modality for all subjects regardless of their FD mutation, and (iii) the potential of lucerastat to reduce the net Gb3 load in tissues to alleviate key symptoms of the disease and delay progression towards end-stage organ failure.

1.3.1 Unmet medical need of subjects with Fabry disease

As available therapies have not yet demonstrated clear long-term clinical benefit there is still a high unmet medical need in FD [see Section 1.1.2]. Additionally, the use of existing therapies is restricted, either due to their mode of administration and immunogenic potential, or due to a treatment modality from which only a subset of subjects with specific FD mutations can benefit.

1.3.2 Substrate reduction therapy with lucerastat as a distinct treatment modality

SRT may be achieved using small-molecule GCS inhibitors such as oral lucerastat, which can be administered daily and lacks the immunogenic potential of recombinant enzymes (i.e., ERT).

SRT with lucerastat has the potential to produce the desired PD effect regardless of the subject's *GLA* mutation, including mutations associated with very low or no residual α-GalA activity [see Section 1.2.2].

SRT with lucerastat was demonstrated in the exploratory Phase 1b study in subjects with FD receiving ERT, as shown by a fast and marked reduction in the plasma concentration of the biomarkers GlcCer, LacCer and Gb3 [see Section 1.2.3 and Lucerastat IB].

In addition, SRT with marketed drugs (miglustat, eliglustat) has proven effective and safe for the treatment of subjects with type 1 Gaucher disease, another GSL storage disorder.

1.3.3 Potential of lucerastat to interfere with the mechanisms underlying key Fabry disease symptoms — neuropathic pain and gastrointestinal symptoms

Typical manifestations of pain and acroparesthesia affecting subjects with FD are thought to result from poor perfusion of the peripheral nerves and lysosomal accumulation of GSL in neurons, DRG and spinal cord, in the form of lamellar inclusions, which may contribute to the atrophy of small unmyelinated nerves [Kaye 1988]. Subjects with FD have severely enlarged DRG [Godel 2017], which may be due to Gb3 accumulation mediating direct neurotoxic effects and decreased neuronal blood supply [Møller 2007, Godel 2017]. The DRG are intensely vascularized organs with high permeability between blood and nervous tissue and, therefore, are expected to be exposed to lucerastat concentrations similar to those in blood [Godel 2016]. SRT with lucerastat was able to reduce Gb3 storage levels in the DRG in a mouse model of FD devoid of α-GalA activity [Lucerastat IB].

Hence, a therapeutic intervention such as SRT with lucerastat would be expected to reduce Gb3 accumulation in neurons, DRG and spinal cord, and mitigate neuropathic pain, one of the most frequent debilitating symptoms of FD, which poorly responds to current pharmaceutical treatments [Alegra 2012, Markham 2016].

Although the pathophysiology of GI manifestations in FD is not fully understood, it is postulated that almost all the cells and tissues contributing to intestinal function and

structure may be affected [Keshav 2006]. Neuropathy, vasculopathy, and myopathy are generally considered to be a major cause of GI symptoms; these alterations are thought to be due to Gb3 accumulation in the vegetative neurons of the autonomic plexus, in the smooth muscle cells as well as in the vascular endothelium [Buda 2013]. Similar to neuropathic pain, a therapeutic intervention such as SRT with lucerastat would be expected to reduce Gb3 in the GI tract, and may also alleviate GI symptoms of FD, e.g., abdominal pain and diarrhea.

1.4 Summary of known and potential risks and benefits

The potential benefits of lucerastat administration, for subjects participating in this study, include a decrease (in frequency and/or intensity) of FD symptoms such as neuropathic pain, GI symptoms (diarrhea, constipation, abdominal pain), and an improvement of their QoL.

The potential risks for subjects participating in this study are related to those based on the nonclinical findings of the study treatment [see Section 1.2.2], discontinuation of ERT [see Section 5.2.3.1], and risks and discomforts associated with some of the study tests and procedures [see Section 7.2].

There is potential risk in both placebo and lucerastat treatment groups that FD symptoms or condition may not get better or may get worse. It should be noted that during the shortage of the ERT agalsidase beta supply between June 2009 and January 2012, no increase in clinical event incidence [Smid 2011] and only mild decrease in renal function [Weidemann 2014, Lenders 2016] could be observed in subjects with FD receiving a reduced dose of agalsidase beta.

In this study, the most invasive procedure repeated at each visit will be blood sampling. All test procedures (e.g., blood sampling, electrocardiogram [ECG], echocardiography) are part of the routine standard-of-care of subjects with FD, although their frequency may be higher in the study. The use of the electronic diary (eDiary) carries no to little risk to the subjects.

The following measures will contribute to minimize the risks for the subjects participating in the study, in particular those who discontinue ERT at screening:

- Exclusion of subjects at high risk of developing clinical signs of organ involvement within the time period of the study (as per investigator judgment), including subjects with substantial organ damage and severe complications of FD [see Section 4.4];
- Maintenance of the background pain medications such as anti-epileptics and TCAs [see Section 5.2.3.2];
- Individualized Pain Management Plan (IPMP) set up at the screening visit for each subject [see Section 5.2.3.2.2];

- Close follow-up of the subjects throughout the study with daily collection of symptoms via an eDiary and with four site visits scheduled during the 6-month treatment period [see Section 7.1];
- Monitoring of safety and efficacy data by an Independent Data Monitoring Committee (IDMC) [see Section 3.3.1];
- Study-specific criteria for stopping study treatment [see Section 5.1.10];
- Study-specific study treatment dose adjustment in the event of a decrease in estimated glomerular filtration rate (eGFR) [see Section 5.1.2];
- Possibility to (re-)initiate ERT if judged medically required due to significant FD progression [see Section 5.2.4.1].

It is the investigator's responsibility to continuously monitor the risk-benefit ratio of the study on an individual subject level, and to take the appropriate measures to ensure the subjects' well-being.

2 STUDY OBJECTIVES

The overall objective of this study is to determine the clinical efficacy of lucerastat oral monotherapy and to further evaluate its safety and tolerability in adult subjects with FD over a period of 6 months.

2.1 Primary objective

The primary objective of the study is to determine the effect of lucerastat on neuropathic pain in subjects with FD.

2.2 Secondary objectives

- To determine the effects of lucerastat on GI symptoms (abdominal pain and diarrhea) in subjects with FD and GI symptom(s) at baseline;
- To confirm the effect of lucerastat on biomarkers of FD;
- To determine the safety and tolerability of lucerastat in subjects with FD.

2.3 Other objectives

- To evaluate the effect of lucerastat on renal function and cardiac parameters in subjects with FD;
- To evaluate the effect of lucerastat on depression in subjects with FD;
- To evaluate the effect of lucerastat on QoL in subjects with FD;
- To document the PK of lucerastat in subjects with FD.

3 OVERALL STUDY DESIGN AND PLAN

3.1 Study design

This is a prospective, multicenter, double-blind, randomized, placebo-controlled, parallel-group, Phase 3 study.

Approximately 99 adult subjects with FD exhibiting Fabry-associated pain of moderate to severe intensity will be randomized in a 2:1 ratio to either lucerastat (approximately 66 subjects) or placebo (approximately 33 subjects).

Treatment allocation will be stratified by sex and by specific background FD treatment at screening (subjects treated with ERT also called "switch" subjects, as they will have to stop ERT at screening visit, vs subjects not treated with ERT at screening). Subjects not treated with ERT at screening include (i) "treatment-naïve" subjects who were never treated with ERT and (ii) "pseudo-naïve" subjects who stopped ERT at least 6 months prior to screening.

The study will be conducted at approximately 58 sites in approximately 14 countries.

Once randomized, the subject will enter a 6-month double-blind treatment period [see Section 3.1.1].

All English speaking subjects in the US and Canada will participate in an exit interview as soon as possible after the end of study treatment phase or End-of-Treatment (EOT) visit, ideally within 2 weeks.

3.1.1 Study periods

The study comprises the following consecutive periods:

Screening period: Lasts approximately 6–7 weeks; starts with the signing of the informed consent form (ICF; at the screening visit) and ends the day before subject randomization.

Treatment period: Lasts approximately 6 months. Starts on the day of subject randomization (randomization visit) and ends at the EOT visit (Month 6). It will consist of site visits at Month 1, Month 3, Month 5 and Month 6 and telephone call visits at Month 2 and Month 4.

The EOT visit will take place at Month 6 (or earlier in the event of premature discontinuation).

Whenever possible, the EOT visit should take place 1 day after the last dose of study treatment but no later than 7 days after the last dose of study treatment.

Post-treatment observation period (PTOP): Subjects who discontinue study treatment prematurely will enter into the PTOP, which starts on the day after the last dose of study treatment, and ends at latest at the Month 6 PTOP visit.

During this period, all assessments except PK will be performed. They will be performed at the time of the originally scheduled visits [Table 6].

Post-treatment safety follow-up (FU) period: The FU period is applicable to all subjects except those who enter the open-label extension (OLE) study. It starts on the day after the last dose of study treatment:

- For female and non-fertile male subjects: it includes 1 safety FU telephone call (FU1) taking place approximately 1 month after the last dose of study treatment;
- For fertile male subjects: it includes 2 safety FU telephone calls taking place approximately 1 month (FU1) and 3 months (FU2) after the last dose of study treatment.

Subjects who complete the 6-month double-blind treatment period will be proposed to enroll into an OLE study conducted under a separate protocol (provided the OLE study protocol has been approved in the country/site by regulatory authorities and Ethics Committees [ECs] / Institutional Review Boards [IRBs]).

Subjects who discontinue study treatment prematurely for any reason should be subsequently treated according to local standard-of-care at the investigator's discretion and will be followed in the PTOP until the originally scheduled Month 6 visit.

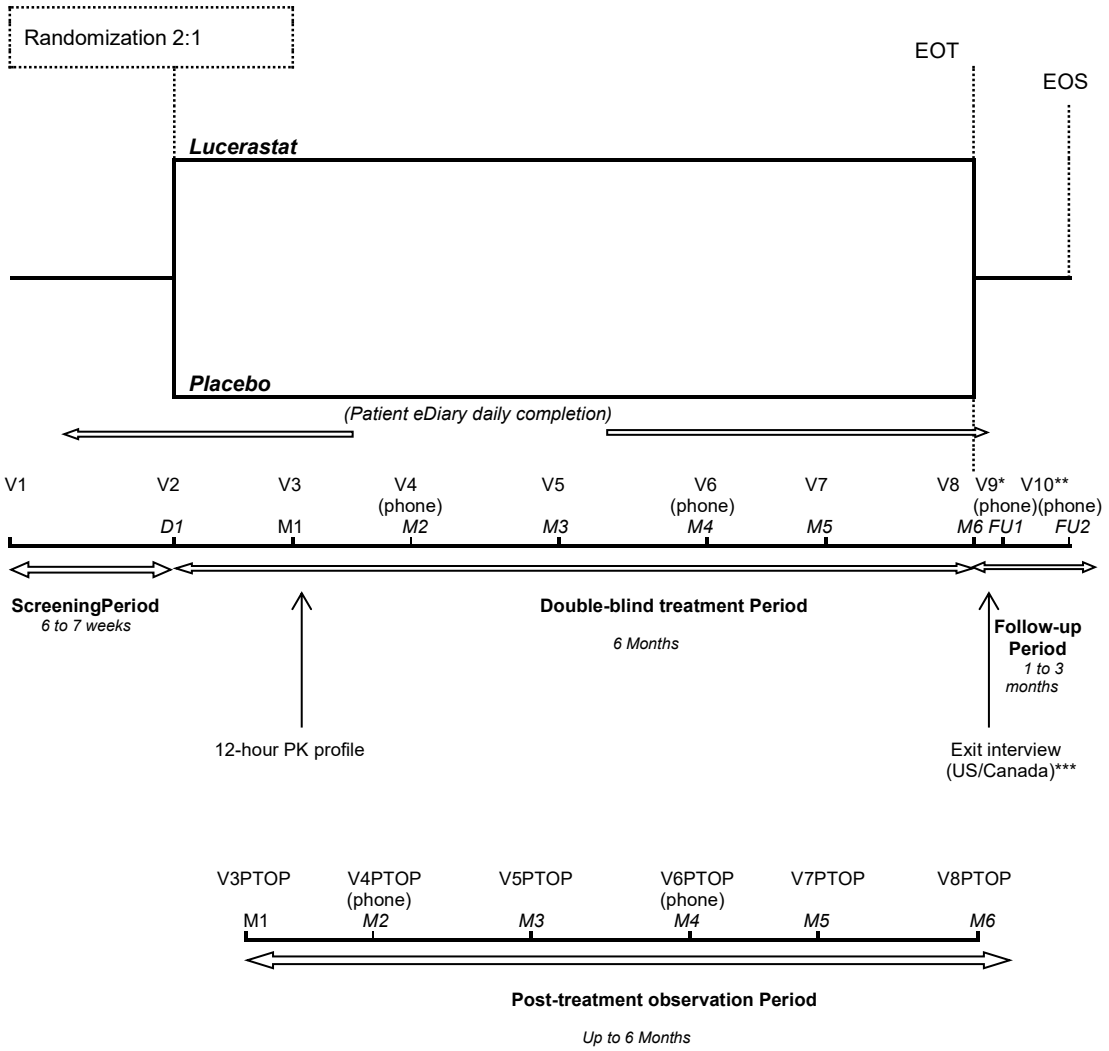
For an individual subject, the End-of-Study (EOS) is defined as follows:

- For subjects who enter the OLE study, the EOS corresponds to the EOT visit;
- For all other subjects, the EOS corresponds to the FU1 visit, FU2 visit or to the last visit of the PTOP, whichever is last.

The visit schedule and protocol-mandated procedures will be performed according to the tables of assessments [Table 5 and Table 6] and are described in Section 7.1.

The overall study design is depicted in [Figure 1].

Figure 1 Study design



* All subjects not entering the OLE study
** Only fertile male subjects not entering the OLE study
*** Exit interview conducted as soon as possible after the end of study treatment phase (or EOT visit), ideally within 2 weeks
D = day; EOS = End-of-Study; EOT = End-of-Treatment; FU = follow-up (telephone); M = Month; OLE = open-label extension; PK = pharmacokinetics; PTOP = post-treatment observation period; V = Visit.

3.1.2 Study duration and global end of the study

The study starts with the first subject, first visit and ends with the last subject, last visit. The study is expected to last approximately 3.5 years.

The duration of individual participation in the study is expected to be about 9 months for a female subject and between 9 and 11 months for a male subject.

The global end of the study corresponds to the last visit of the last subject:

- If the last subject enters the OLE study, the global end of the study corresponds to the last subject's EOT visit.
- If the last subject does not enter the OLE study, the global end of the study corresponds to the last subject's FU1 visit, FU2 visit, or to the last visit of the PTOP, whichever is last.

3.1.3 Sub-studies

As part of this protocol, the following sub-studies will be performed:

3.1.3.1 Pharmacokinetic profile sub-study (all sites)

The purpose of the PK sub-study is to further characterize the PK profile of lucerastat. It will be conducted at all sites to determine the PK profile of lucerastat at steady state in a subgroup of approximately 36 subjects with FD with either normal or subnormal renal function (i.e., screening eGFR $\geq$ 60 mL/min/1.73 m^2) or impaired renal function (i.e., screening eGFR < 60 mL/min/1.73 m^2). If necessary, blood samples collected for PK may be used for the identification of potential metabolites of lucerastat in this population.

The enrollment in the sub-study will be managed by the Interactive Response Technology (IRT) system and will continue in each of the eGFR subgroups until 18 subjects (12 subjects randomized to lucerastat and 6 randomized to placebo) are enrolled in each subgroup. If the PK sub-study is not fully enrolled when the recruitment target at overall study level (i.e., 108 subjects) is reached, the PK sub-study will not continue recruiting.

Subjects consenting to this sub-study will have a 12-hour PK profile performed at the Month 1 visit. Further details on PK sampling and collection are available in Section 7.2.6.2. PK profile endpoints are defined in Section 6.4.1.

3.1.3.2 Exit interview sub-study (US and Canadian sites only)

The purpose of the exit interview sub-study is to further explore what constitutes meaningful changes on the neuropathic pain and GI symptom (diarrhea and abdominal pain) questions from a subject perspective.

The exit interview sub-study will be conducted in all English-speaking subjects at US and Canadian sites.

Details on the analysis of the exit interview data are available in the Exit Interview Analysis Plan.

Subjects will be contacted by a Contract Research Organization (CRO) to set an interview date as soon as possible after the end of study treatment phase (or EOT visit), ideally within 2 weeks. All interviews will be conducted via telephone and will take approximately 45 minutes to complete.

More details are available in the sub-study Protocol and Discussion Guide for the Exit Interview which have been developed separately.

3.2 Overall study design rationale

Neuropathic pain and GI symptoms are important clinical manifestations of FD.

In the absence of regulatory guidelines specific to the development of treatment for FD at the time of the first protocol version, the methodology used in the study to assess the effect of lucerastat on neuropathic pain and GI symptoms has been based whenever applicable on the Committee for Medicinal Products for Human Use (CHMP) guideline for neuropathic pain [CHMP 2007] (later replaced by the CHMP guideline on pain [CHMP 2017], and FDA guidance on chronic pain [FDA 2014], irritable bowel syndrome (IBS) [FDA 2012], and Patient Reported Outcome (PRO) [FDA 2009]. In particular, those guidelines recommend the use of a randomized, double-blind study design when assessing subject symptoms using a PRO. In 2019, FDA issued a draft FDA guidance on development of drugs for FD treatment [FDA 2019]. The overall study design and endpoint chosen are in line with this guidance.

Due to a high and variable placebo response rate in pain trials (i.e., a systematic tendency for efficacy measures to show an improvement from baseline to endpoint of the trial irrespective of treatment allocation) placebo controlled superiority trials are necessary [CHMP 2017, FDA 2012].

Both the FDA and the CHMP [FDA 2014] and [CHMP 2017] recommend at least a 3-month duration for studies evaluating analgesia in a chronic pain condition. With its mode of action, lucerastat is being developed as a disease-modifying drug for subjects with FD. It is postulated that the potential effects of lucerastat on the established clinical symptoms of neuropathic pain will result from its PD effect, i.e., the reduction of Gb3 and lysoGb3 storage in the DRG and peripheral nerves [see Section 1.3]. This “de-bulking” effect is likely to require a longer time period of treatment than drugs having a direct central analgesic effect. In a mouse model of FD, the effect of oral lucerastat on the storage of α-GalA substrates including Gb3 in DRG was greater after 20 weeks of treatment (48% reduction) compared to 10 weeks of treatment (27% reduction) [Lucerastat IB]. In subjects with FD who received 1000 mg b.i.d. lucerastat on top of ERT, there was a fast onset of the effect of lucerastat on plasma levels of FD biomarkers with a mean percentage

change from baseline (SD) of –50% (14), –38% (8), and –39% (14) in plasma levels of GlcCer, LacCer, and Gb3, respectively, at Week 4 [Lucerastat IB]. Based on those data, it is expected that the effect would already be measurable at Month 6.

Temporary interruption of ERT for a maximum of 8 months in subjects with neuropathic pain is acceptable for the following reasons:

- FD is a slowly progressive disease with respect to clinical signs and symptoms, and organ function impairment [see Section 1.1]. Therefore, significant clinical worsening of organ disease in subjects who discontinue ERT is not expected within the time frame of the study;
- ERT stopping criteria recommended by the European Fabry Working Group include the lack of response when the sole "indication" for ERT is neuropathic pain, and the subject's request to stop ERT [Biegstraaten 2015];
- Possibility to (re-)initiate ERT if judged medically required due to significant FD progression [see Section 5.2.4.1].

In addition, several measures are implemented for safeguarding the interests of study subjects as described in Section 1.4.

The choice of the stratification factors (sex and ERT treatment status at screening) was primarily based on (i) the difference in the age of onset and neuropathic pain history observed between male and female subjects [Mehta 2004, Deegan 2006] and (ii) the possibility that the time-course of the placebo-corrected effect of lucerastat on neuropathic pain may be different between "switch" subjects and "pseudo-naïve" / "treatment-naïve" subjects.

3.3 Study committee

3.3.1 Independent Data Monitoring Committee

An IDMC will have overall responsibility for safeguarding the interests of subjects by monitoring safety and efficacy data obtained in the study and making appropriate recommendations based on the reported data, thus ensuring that the study is being conducted to the highest scientific and ethical standards. The IDMC will be fully operational prior to enrollment of the first subject into the study. The composition and operation of the IDMC will be described in an IDMC charter.

4 STUDY POPULATION

4.1 Selection of study population

In general, diagnosis of FD combines clinical presentation, α-GalA activity and *GLA* mutations, as defined in the most recent international guidelines [Schiffmann 2017]. This study will specifically enroll adult male and female subjects with a diagnosis of FD, as

confirmed by the presence of at least one *GLA* gene mutation, with Fabry-associated neuropathic pain in the last 3 months prior to screening.

Neuropathic pain in FD usually feels like burning, shocks or shooting pain, tingling, pins and needles, stabbing, and/or numbness in the hands and feet. The patient may experience constant pain of variable intensity. He/she may also get attacks of intense, excruciating pain that starts in the hands and feet and spreads out to other parts of the body. Pain may occur randomly. It may also be triggered by heat or cold, weather change, being sick or having a fever, and/or physical activity [Biegstraaten 2012, Politei 2016, Üceyler 2014].

The subjects will have to present with moderate to severe neuropathic pain resulting in an average modified BPI-Short Form item 3 (BPI-SF3) of "neuropathic pain at its worst in the last 24 hours" ≥ 4 as reported on an 11-point numerical rating scale (NRS-11) during the last 4 weeks preceding randomization.

Participation in the study will be proposed to subjects with FD for whom continuation or initiation of ERT is not medically indicated, as determined by the investigator, based on the medical history of the subject and in accordance with local or international treatment guidelines [Biegstraaten 2015, Politei 2016, Schiffmann 2017]. Subjects at high risk of developing clinical signs of organ involvement within the time period of the study, including subjects with substantial organ damage and severe complications of FD are not eligible for the study [Section 4.4].

Subjects who are currently treated with ERT must agree to stop ERT at the screening visit (i.e., "switch" subjects). Subjects who are "treatment-naïve" (i.e., no ERT received at any time in the past), or "pseudo-naïve" (i.e., no ERT received in the last 6 months prior to screening) must not be planned for imminent initiation of ERT at the time of enrolment.

Subjects with significant medical conditions or therapies for such conditions: cardiovascular, renal or cerebrovascular and subjects with any disease that could interfere with the interpretation of the study results (e.g., disease with pain components) are not eligible to enter the study [see Section 4.4].

In order to minimize the rate of screening failures, the site is encouraged to perform pre-screening activities by, e.g., reviewing subject charts. Subjects with any of the chronic conditions mentioned in the exclusion criteria list [see Section 4.4] should not be considered for screening.

4.2 Rationale for the selection of the study population

The targeted FD study population is homogeneous with regards to FD-associated neuropathic pain (as defined by the subject) in the last 3 months prior to screening, and the presence of moderate to severe neuropathic pain during the 4 weeks preceding

randomization as determined from daily entries of the modified BPI-SF3 score in an eDiary.

Published guidelines on pain recommend studying the efficacy in a population that is homogeneous with respect to either diagnosis or pain intensity and to stratify according to baseline disease characteristics [CHMP 2017].

The inclusion of subjects with moderate to severe pain (i.e., worst pain intensity score ≥ 4 reported on an NRS-11) is in line with the recommendations of the CHMP guidelines [CHMP 2017] and justified by the higher placebo effect expected in a population with mild pain (i.e., worst pain intensity score < 4 reported on an NRS-11). Furthermore, mild neuropathic pain typically causes the least interference with "function" and most often only requires mild analgesic treatment (e.g., acetaminophen) [Cleeland 2002]. Therefore, subjects with mild neuropathic pain will be excluded from the study.

The goal of MODIFY is to determine the effect of lucerastat as an oral monotherapy in subjects with FD. At the time of screening, subjects could either be off ERT or on ERT but willing to stop it. Indeed, a significant proportion of subjects with FD still exhibit symptoms of neuropathic pain despite long-term treatment with ERT. The European Fabry Working Group has published a recommendation for cessation of ERT in patients with FD [Biegstraaten 2015]. Consensus criteria to stop ERT include the lack of response for 1 year when the sole "indication" for ERT is neuropathic pain, non-compliance for more than 50% of the ERT infusions, and the patient's request to stop ERT. Subjects on ERT at screening can only be enrolled if they fulfil the neuropathic pain inclusion criteria despite exposure to ERT for at least 12 months.

The age range (i.e., 18 years or above) of the study population is representative of the general adult FD population.

Subjects with major cardiovascular, renal, cerebrovascular medical complications or other diseases with pain components are excluded since they could potentially be at greater risk of experiencing side effects, and/or their conditions could interfere with evaluation of the treatment effect, study assessment and interpretation of study results.

4.3 Inclusion criteria

For inclusion in the study, the subject must fulfill all the following inclusion criteria at the specified study visits. It is not permitted to waive any of the criteria for any subject:

Screening visit criteria

1. Signed and dated ICF prior to any study-mandated procedure;
2. Male or female subjects 18 years old and above;

3. FD diagnosis confirmed with local genetic test results (i.e., presence of at least 1 mutation in *GLA,* the gene coding for α-GalA);
4. Fabry-associated neuropathic pain, as defined by the subject, in the last 3 months prior to screening;
5. ERT treatment status:

   a) Subject never treated with ERT; or

   b) Subject has not received ERT for at least 6 months prior to screening; or

   c) Subject treated with ERT at the time of the screening visit, and meeting all of the following criteria at the time of screening:

      i) ERT administration for the last 12 months;

      ii) Stable ERT dose regimen during the last 3 months;

      iii) Subject agrees to stop ERT administration at the screening visit for approximately 8 months (6–7 weeks screening + 6 months of double-blind treatment).
6. A woman of childbearing potential [see definition in Section 4.5.1] is eligible only if the following applies:

   - Negative serum pregnancy test at screening and a negative urine pregnancy test at randomization;
   - Agreement to undertake monthly urine pregnancy tests during the study and up to at least 30 days after study treatment discontinuation;
   - Agreement to follow a highly effective contraception scheme as described in Section 4.5.2 from screening up to at least 30 days after study treatment discontinuation.
7. A fertile male (physiologically capable of conceiving a child according to investigator judgment) who is sexually active with a woman of childbearing potential is eligible only if the following applies:

   - Agreement to use a condom during the treatment period (starting at randomization) and for up to 3 months after study treatment discontinuation; and
   - Agreement not to father a child during this period.

Randomization visit criteria

8. Adequate subject compliance with completion of an eDiary during the screening period;

9. Subjects with moderate or severe neuropathic pain as determined from daily entries of the modified BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours" in the eDiary during the screening period.

Moderate or severe neuropathic pain during the screening period is defined as an average modified BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours" ≥ 4 as determined from daily entries in an eDiary during the 4 weeks preceding randomization.

Adequate subject compliance with completion of the eDiary is defined as the availability of the modified BPI-SF3 score of at least 4 days per week, in at least 3 out of the 4 weeks preceding randomization.

4.4 Exclusion criteria

Subjects must not fulfill any of the following exclusion criteria at the specified study visits. It is not permitted to waive any of the criteria for any subject:

Screening visit criteria

Disease/condition

1. Pregnant / planning to become pregnant up to 30 days after study treatment discontinuation or lactating subject;
2. Severe renal insufficiency defined as an eGFR per the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation < 30 mL/min/1.73 m^2 at screening (as reported by the central laboratory);
3. Subject on regular dialysis for the treatment of chronic kidney disease;
4. Subject has undergone, or is on a waiting list for, or is scheduled to undergo kidney or other organ transplantation;
5. Known and documented transient ischemic attack, stroke, unstable angina or myocardial infarction within 6 months prior to screening;
6. Clinically significant unstable cardiac disease in the opinion of the investigator (e.g., uncontrolled symptomatic arrhythmia, New York Heart Association [NYHA] class III or IV congestive heart failure);
7. Any other subject at high risk of developing clinical signs of organ involvement within the time period of the study, as per investigator judgment;
8. Any known factor or disease that might interfere with treatment compliance, study conduct or interpretation of the results such as:

   a) Other disease or condition associated with a pain component that could confound assessment of neuropathic pain (e.g., diabetic neuropathy, chemotherapy- or

radiation-induced peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy);

b) Other disease of the GI tract that could interfere with the assessment of GI symptoms in FD (e.g., inflammatory bowel disease, galactose intolerance, total lactase deficiency or glucose-galactose malabsorption);

c) Documented poorly controlled diabetes mellitus (i.e., HbA1c > 8.0% at screening as reported by the central laboratory);

d) Significant neurological disorder;

e) Significant psychiatric disease; suicidal ideation at screening or history of suicide attempt or behavior within 6 months prior to screening as per investigator judgment;

f) History of drug dependence (including opioids) or alcohol dependence;

g) Inability to complete an eDiary on a daily basis.

9. Known concomitant life-threatening disease with a life expectancy < 18 months.

Treatments

10. Subject planned for imminent initiation of treatment with ERT;
11. Known hypersensitivity to lucerastat or drug of the same chemical class of iminosugars (e.g., miglitol, miglustat, migalastat), or any of their excipients (including lactose);
12. Initiation or treatment at an unstable dose within 4 weeks prior to screening with any of the following medications:

    a) ACE inhibitor and/or ARB;

    b) Anti-epileptic;

    c) TCA and/or other antidepressants belonging to SNRI and selective serotonin re-uptake inhibitor (SSRI) classes.

13. Planned or current treatment with another investigational treatment within 3 months prior to screening;
14. Treatment with any inhibitor of GCS (e.g., miglustat, lucerastat, eliglustat, ibiglustat/venglustat) or an α-GalA chaperone (e.g., migalastat) within 6 months prior to screening;

Randomization visit criteria

15. Treatment with ERT (agalsidase alfa, agalsidase beta) during the screening period.

In addition, the investigator/delegate must verify that the subject does not fulfill the exclusion criteria checked at the screening visit (as applicable).

4.5 Contraception requirements for women of childbearing potential

4.5.1 Definition of childbearing potential

A woman is considered to be of childbearing potential unless she meets at least 1 of the following criteria:

- Previous bilateral salpingectomy, bilateral salpingo-oophorectomy or hysterectomy;
- Postmenopausal (defined as 12 consecutive months with no menses without an alternative medical cause [ICH M3 definition]);
- Premature ovarian failure (confirmed by a specialist), XY genotype, Turner syndrome, uterine agenesis;

The reason for not being of childbearing potential will be recorded in the electronic case report form (eCRF) and hospital charts.

4.5.2 Highly effective methods of contraception

Women of childbearing potential [see definition in Section 4.5.1] must follow a highly effective [CTFG 2014] contraception scheme from screening up to at least 30 days after study treatment discontinuation as follows:

1. Two methods of contraception, one from Group 1 and one from Group 2, defined as follows:
   - **Group 1:** Oral, implantable, intravaginal, transdermal, or injectable hormonal contraceptives. If a hormonal contraceptive is chosen from this group, it must be taken for at least 1 month prior to randomization;
   - **Group 2:** Partner's use of a condom (preferred method), female condom, cervical cap or diaphragm. Cervical cap and diaphragm must be used in combination with a spermicide. In countries where spermicide is not available/authorized, cap and diaphragm must not be used.

OR

2. Intrauterine device or intrauterine hormone-releasing system;

OR

3. Sterilization of the male partner with documented post-vasectomy confirmation of the absence of sperm in the ejaculate;

OR

4. Tubal sterilization (tubal occlusion / ligation at least 6 weeks prior to screening);

OR

5. True abstinence from intercourse with a male partner only when this is in line with the preferred lifestyle of the subject and if locally accepted as a reliable method of contraception.

The following methods are not allowed as methods of contraception for this study:

- Progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- Periodic abstinence (calendar, symptothermal, post-ovulation methods)
- Withdrawal (coitus interruptus)
- Spermicides only
- Lactational amenorrhea method
- Combination of female condom and male condom
- Use of only Group 2 “barrier contraception method” (see above)

The methods of birth control used (including non-pharmacological methods) must be recorded in the eCRF and hospital charts.

To ensure compliance, the study personnel must remind women of childbearing potential at each visit to use the methods of contraception defined for this study. The reminders must be documented in the hospital chart.

4.6 Contraception requirements for fertile males

A fertile male is defined as physiologically capable of conceiving child according to the investigator’s judgment.

Fertile male subjects participating in the study who are sexually active with women of childbearing potential must agree to use a condom during the treatment period and for up to 3 months after study treatment discontinuation and agree to not father a child during this period.

To ensure compliance, the study personnel must remind the subjects of the contraception requirements at each visit, and ensure the reminders are documented in the hospital chart.

5 TREATMENTS

5.1 Study treatment

5.1.1 Investigational treatment and matching placebo: description

Lucerastat is available for clinical study use in hard gelatin capsules containing 250 mg of lucerastat and inactive excipients ([redacted] mg of lactose anhydrous and [redacted] mg of talc).

Matching placebo capsules will contain inactive excipients ([redacted] mg of lactose anhydrous and [redacted] mg of talc).

The maximum amount of lactose ingested by a subject enrolled in the placebo group will be [redacted] mg per dose and [redacted] mg per day.

5.1.2 Study treatment dosing and administration

Subjects will take either lucerastat or matching placebo orally b.i.d.

The starting dose of the study treatment (lucerastat or matching placebo) will be based on the subject's eGFR value (as reported by the central laboratory) at the screening visit as shown in Table 1.

During the study, the dose of the study treatment will be reduced if the subject's eGFR (as reported by the central laboratory during scheduled or unscheduled visits) decreases and crosses the next lower eGFR boundary as shown in Table 1. Upon receipt of the eGFR results from the central laboratory, the investigator/delegate will contact the subject to provide instruction if a dose adjustment is required. The date of the contact with the subjects and the reduced dose will be collected in the eCRF.

Study treatment must be discontinued if one of the study-treatment stopping criteria is met [see Section 5.1.10].

Table 1 eGFR-based study treatment dosing scheme

eGFR (mL/min/1.73 m^2)	Dose regimen (oral)	Number of capsules per dosing
≥ 60	1000 mg b.i.d.	4
≥ 45 and < 60	750 mg b.i.d.	3
≥ 30 and < 45	500 mg b.i.d.	2
≥ 15* and < 30	250 mg b.i.d.	1

* study treatment must be stopped if eGFR < 15 mL/min/1.73m^2 or in the event that the *acute kidney injury* CTCAE grade 2 or above is met, [see Section 5.1.10].
b.i.d. = twice daily; CTCAE = Common Terminology Criteria for Adverse Events; eGFR = estimated glomerular filtration rate.

Subjects will be instructed to start taking the study treatment on the evening of the randomization visit. Thereafter, the subject should take each study treatment dose in the morning and evening irrespective of food intake. It is preferable that the study treatment be taken each day (morning and evening) at approximately the same time.

Subjects will be instructed not to take study treatment in the morning of study visit days. On the day of the study visits, study treatment must be administered only after the completion of the pre-dose assessments as indicated in Table 5.

If a dose is missed, it should be taken as soon as possible. However, the missed dose should be skipped if it is almost time for the next dose.

To ensure compliance, the study personnel must remind subjects at each visit of the study treatment intake requirements. The reminders must be documented in the hospital chart.

5.1.3 Treatment assignment

A total of 99 eligible subjects will be randomized in a 2:1 ratio to lucerastat or placebo, stratified by sex (male, female) and by ERT treatment status at screening (treated, “pseudo-naïve” / “treatment-naïve”).

At the randomization visit, and after having verified that the subject meets all inclusion criteria and none of the exclusion criteria, the investigator/delegate contacts the IRT system to randomize the subject.

The investigator/delegate will report the screening visit eGFR value of the subject (as reported by the central laboratory) in the IRT system. The IRT system assigns a randomization number to the subject and assigns the treatment kit numbers, which match the treatment arm assigned per the randomization list. The number of treatment kits assigned to the subject will depend on the study treatment dose established for the subject [see Section 5.1.2].

The IRT system is handled by an external independent vendor which will generate the randomization list.

5.1.4 Blinding

This study will be performed in a double-blind fashion. The investigator, study personnel, subjects, Clinical Research Associates (CRAs), sponsor personnel, and vendor/CRO personnel involved in the conduct of the study will remain blinded to the study treatment received by the subjects during the double-blind treatment period until study closure.

To ensure adequate supply of study treatment, the IRT vendor personnel responsible for clinical study supply distribution and the sponsor individuals contributing to clinical supply distribution will need to be unblinded at subject level and depot level, respectively. These

persons will be clearly identified, their unblinding will be documented in the trial master file, and they will not take part in any Clinical Trial Team (CTT) meetings after study set-up has been completed.

Until the time of unblinding for final data analysis, the randomization list is kept strictly confidential, and accessible only to IRT vendor and sponsor authorized persons (i.e., Pharmaceutical Development group, Bioanalytical Laboratory group), who are not involved in the conduct of the study.

The investigational treatment and its matching placebo are indistinguishable, and all treatment kits will be packaged in the same way.

To minimize the possibility of systematic unblinding, the results of the biomarker [see list in Section 7.2.7.1] and PK data will not be communicated to the investigator, study personnel, subjects, CRAs, any sponsor or vendor/CRO personnel involved in the conduct of the study. Results will be transferred by the central laboratory (for biomarkers) and the sponsor Bioanalytical Laboratory group (for PK data) to the sponsor and CRO personnel involved in the conduct of the study only after database lock.

5.1.5 Unblinding

5.1.5.1 Unblinding for final analyses

Full randomization information will be made available for data analysis only after database lock, in accordance with the sponsor's Quality System (QS) documents.

5.1.5.2 Unblinding for suspected unexpected serious adverse reactions

If a suspected unexpected serious adverse reaction (SUSAR) [see definition in Section 9.1.3] occurs in a subject participating in the study, the sponsor's Global Drug Safety department will request the unblinding of the treatment assignment in order to meet regulatory reporting requirement.

The treatment assignment will not be communicated to site personnel, subjects, sponsor CTT or any vendor/CRO personnel involved in the conduct of the study.

Unblinded SUSAR information will be reported to respective health authorities and Independent ECs (IECs) / IRBs only. SUSARs will be notified to investigators in a blinded fashion.

5.1.5.3 Emergency procedure for unblinding

The investigator, study personnel, subjects, CRAs, sponsor personnel, and any CRO personnel involved in the conduct of the study must remain blinded to the subject's treatment assignment.

The identity of the study treatment may be revealed only if the subject experiences an emergency medical event, the management of which would require knowledge of the blinded treatment assignment. In this case, the decision to unblind resides solely with the investigator and the investigator can receive the unblinded treatment assignment through the IRT system. Whenever possible, and provided it does not interfere with (or does not delay) any decision in the best interest of the subject, the investigator is invited to discuss the intended unblinding with the sponsor personnel.

The occurrence of any emergency unblinding during the study must be clearly justified and explained by the investigator. In all cases, the sponsor personnel must be informed about the emergency unblinding as soon as possible before or after the unblinding.

The circumstances leading to unblinding must be documented in the hospital charts, the Investigator Site File (ISF) and in the eCRF.

5.1.6 Study treatment supply

Manufacturing, labeling, packaging, and supply of study treatment will be conducted according to Good Manufacturing Practice, GCP, and any local or national regulatory requirements.

All study treatment supplies are to be used only in accordance with this protocol, and not for any other purpose.

5.1.6.1 Study treatment packaging and labeling

Study treatment is provided as capsules in childproof bottles.

Study treatment is labeled to comply with the applicable laws and regulations of the countries in which the study sites are located.

5.1.6.2 Study treatment distribution and storage

Study treatment supplies must be kept in an appropriate, secure area and stored according to the conditions specified on the label and in the IB [Lucerastat IB].

5.1.6.3 Study treatment dispensing

The subjects will receive sufficient study treatment to cover the period up to the next scheduled visit. Subjects are asked to return all used, partially used, and unused study treatment bottles at each visit. Should the treatment bottle dispensed at a scheduled visit be lost or damaged, a replacement bottle or kit can be requested via the IRT system.

In exceptional circumstances, the investigator/delegate may contact the IRT system shortly prior to the scheduled visit (with the exception of the randomization visit [see Section 5.1.3]) to obtain the treatment kit number(s) to be dispensed at the visit. The protocol-mandated study treatment dispensing procedures may not be altered without

prior written approval from the sponsor. In exceptional circumstances (e.g., if the subject lost the study treatment between two visits, or if the subject is unable to return to the site due to a medical emergency / hospitalization at another hospital), unscheduled dispensing and delivery of study treatment may occur outside of a scheduled visit. An accurate study treatment record of the date and amount of study treatment dispensed to each subject must be available at the site for inspection at any time.

5.1.6.4 Study treatment return and destruction

The protocol-mandated study treatment return procedures may not be altered without prior written approval from the sponsor. On an ongoing basis and/or on termination of the study, the CRA will collect used and unused treatment kits, which will be sent to the warehouse, where the sponsor personnel or a deputy will check treatment reconciliation. In certain circumstances, used and unused study treatment containers may be destroyed at the site once study treatment accountability is finalized and has been checked by the sponsor personnel or the deputy, and written permission for destruction has been obtained from the sponsor.

5.1.7 Study treatment accountability and compliance with study treatment

5.1.7.1 Study treatment accountability

The inventory of study treatment dispensed to and returned by the subject (i.e., study treatment accountability) must be performed by site personnel on the day of the visit and before dispensing further study treatment. It is to be recorded by site personnel on the study treatment dispensing and accountability log and in the eCRF, and checked by the CRA during site visits and at the end of the study. The study treatment accountability log in the eCRF will include at least the following information for each study treatment unit (i.e., bottle) dispensed to the subject:

- Dispensed bottle ID number;
- Date dispensed / number of capsules dispensed (pre-populated in eCRF);
- Date returned / number of capsules returned.

All study treatment supplies, including partially used or empty bottles must be retained at the site until it is verified by the CRA.

If the subject omits to bring the remaining study treatment to a study visit, he/she must be instructed to not take any capsules from the remaining study treatment bottle and to return it at the next visit.

5.1.7.2 Study treatment compliance

Treatment compliance will be assessed based on eDiary data.

The study treatment compliance with the prescribed study treatment dose regimen will be assessed by the sponsor based on eDiary data. On each day, subjects will be asked to enter in the eDiary the number of capsules taken in the morning and evening. The subjects will receive a reminder alarm (through the device) to take their morning and evening doses of study medication.

For each time interval between two visits (i.e., "period"), the eDiary based study treatment compliance will be calculated by the sponsor using the following formula:

eDiary based study treatment compliance = Total number of times the subject took study treatment at the prescribed dose regimen during the period / Total number of times the study treatment should have been taken at the prescribed dose regimen during the period × 100

The prescribed starting dose corresponds to the dose assigned to the subject by the IRT system at randomization visit. In the event of study treatment adjustment [see Section 5.1.2], the prescribed dose corresponds to the new study treatment dose. The starting date of the new study treatment dose will be the date when the investigator/delegate contacts the subject to provide him/her instructions for study treatment adjustment.

The period is defined as the number of days between visit n (or date of first study treatment intake if randomization visit) and visit n+1 (or date of last study treatment intake if EOT visit).

The number of times the study treatment should have been taken at the prescribed dose regimen in a given period is calculated as follows:

- Number of days in the period × 2

Study treatment interruption(s) will be ignored when calculating the study treatment compliance based on the eDiary.

Between visits, study treatment compliance based on the eDiary is expected to be at least 80%. The sponsor will inform the site staff and CRA about any compliance values below 80%. Compliance values below 80% without a medical justification (e.g., AE) and not related to an eDiary completion issue will be considered as a protocol deviation, which will be reported as such to the sponsor by the CRA. In such cases, the investigator must discuss and clarify the reasons for non-compliance with the subject and take appropriate actions to avoid re-occurrence.

5.1.8 Study treatment dose adjustments and interruptions

Study treatment dose adjustment, other than described in Section 5.1.2 is prohibited.

Study treatment may be temporarily interrupted in response to an AE, or other reasons (e.g., diagnostic or therapeutic procedure, study treatment forgotten).

If study treatment is interrupted by the subject for any reason, he/she must immediately inform the investigator.

Interruptions of study treatment must be kept as short as possible.

All study treatment interruptions of at least 2 consecutive days and/or due to an AE must be recorded in the eCRF.

5.1.9 Premature discontinuation of study treatment

The decision to prematurely discontinue study treatment may be made by the subject, the investigator, or sponsor personnel. The main reason (e.g., AE, lack of efficacy, study terminated by sponsor) must be documented in the eCRF.

A subject has the right to prematurely discontinue study treatment at any time, without any justification, by withdrawing from study treatment only or by withdrawing from any further participation in the study (i.e., premature withdrawal from the study [see Section 8.2]). Although a subject is not obliged to give his/her reason for prematurely withdrawing from the treatment or the study, it is recommended that the investigator makes a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

The investigator must discontinue study treatment for a given subject if, on balance, he/she believes that continued administration would be contrary to the best interests of the subject.

Study-specific criteria for discontinuation of study treatment are described in Section 5.1.10.

A subject who prematurely discontinues study treatment is **NOT** considered as withdrawn from the study and will be followed in the PTOP, provided that the subject's consent for this participation in the study has not been withdrawn. Assessments including safety follow-up that are to be performed at each site visit are described in Section 7.1, Table 5 and Table 6.

A subject who prematurely discontinues study treatment and withdraws consent to participate in any further study assessments is considered as withdrawn from the study. Subjects who die or are lost to follow-up are also considered as withdrawn from the study. Withdrawal from the study and follow-up medical care of subjects withdrawn from the study are described in Sections 8.2 and 8.4, respectively.

5.1.10 Study-specific criteria for interruption / premature discontinuation of study treatment

5.1.10.1 Pregnancy

If a subject becomes pregnant while on study treatment, study treatment **must** be permanently discontinued. The investigator/delegate must counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus.

5.1.10.2 Cardiovascular event

Subjects **must** be permanently discontinued from study treatment at any time during the study in the event of heart failure leading to in-patient hospitalization or prolongation of ongoing hospitalization.

5.1.10.3 Renal function

Subjects **must** be permanently discontinued from study treatment at any time during the study if eGFR < 15 mL/min/1.73m^2 or in the event of acute kidney injury Common Terminology Criteria for Adverse Events (CTCAE) grade 2 or above (i.e., creatinine > 2 × baseline creatinine, creatinine > 4.0 mg/dL or 353.6 µmol/L, hospitalization indicated, life-threatening consequences, dialysis indicated) [CTCAE 2010].

5.1.10.4 Cerebrovascular event

Subjects **must** be permanently discontinued from study treatment at any time during the study in the event of stroke CTCAE grade 3 or above (i.e., severe neurological deficit, life-threatening consequences, urgent intervention indicated) [CTCAE 2010].

5.2 Previous and concomitant therapy

5.2.1 Definitions

A previous therapy is any treatment for which the end date is prior to the signature of the ICF.

A therapy that is study-concomitant is any treatment that is ongoing or initiated after the signature of the ICF, or initiated up to 30 days after study treatment discontinuation.

A therapy that is study treatment-concomitant is any treatment that is either ongoing at the start of study treatment or is initiated during the study treatment period.

Local or international treatment guidelines [Biegstraaten 2015; Politei 2016; Schiffmann 2017] for the management of subjects with FD will apply whenever relevant to the study. These guidelines include the use of ERT and adjunctive therapies.

5.2.2 Mandatory concomitant therapy

Mandatory therapy includes any treatments required for contraception purposes in women of childbearing potential.

5.2.3 Allowed concomitant therapy

Treatments considered necessary for the subject's well-being and not categorized as forbidden concomitant medications are allowed during the study and must be documented in the medical charts.

5.2.3.1 Angiotensin-converting enzyme inhibitor or angiotensin receptor blocker

Subject must not have initiated ACE inhibitor or ARB therapy within 4 weeks prior to screening. ACE inhibitor or ARB therapy, if used by the subject, must be at a stable dose for at least 4 weeks prior to screening. During the study, initiation or dose adjustment are allowed based on investigator judgment.

5.2.3.2 Pain medications

During the study, the use of adjuvant pain medications, non-opioid and opioid analgesics is allowed if judged medically required by the investigator. Initiation or dose adjustment of pain medications should be done as described in Section 5.2.3.2.2.

5.2.3.2.1 Pain medications used in Fabry disease

A list of pain medications used in FD is provided in Table 2.

Subject must not have initiated adjuvant pain medications (i.e., anti-epileptics, TCAs and SNRIs/SSRIs) within 4 weeks prior to screening. The dose regimen of adjuvant pain medications used chronically by the subject at screening must be stable for at least 4 weeks prior to screening.

Table 2 List of pain medications used in Fabry disease

Categories	Pain medications
Adjuvant pain medications	• Anti-epileptics: e.g., carbamazepine, gabapentin, phenytoin, pregabalin, oxcarbazepin, lamotrigine, topiramate; • TCAs: e.g., amitriptyline, nortriptyline; • Other antidepressants (SNRIs/SSRIs): e.g., duloxetine, venlafaxine.
Non-opioid analgesics	• NSAIDs: e.g., acetylsalicylic acid / aspirin, paracetamol/acetaminophen, ibuprofen, naproxen, diclofenac, metamizole; • Topical analgesics: e.g., lidocaine, prilocaine, capsaicin.
Opioid analgesic	• "Weak" opioids: e.g., codeine, dihydrocodeine; • "Strong" opioids: e.g., morphine, oxycodone, tramadol, fentanyl, methadone, hydromorphone, mepiridine.

NSAID = non-steroidal anti-inflammatory drug; SNRI = serotonin-norepinephrine re-uptake inhibitor; SSRI = selective serotonin re-uptake inhibitor; TCA = tricyclic antidepressant.

5.2.3.2.2 Rescue medications for unbearable pain

In the event of unbearable pain, rescue pain medications may be considered. An IPMP will be provided by the investigator/delegate to each subject with customized instructions regarding the use of pain medications during the study.

The IPMP will list the pain medications ongoing at the screening visit that the subject should continue taking during the study. It will also include a list of new pain medication(s) or dose adjustment that should be made in the event of unbearable pain taking into account the subject's background pain medication(s) and previous response to pain medication(s). The IPMP will be applicable starting at screening visit and until the EOT visit or the last visit of the PTOP (as applicable). The IPMP will also include instructions regarding the need to contact the investigator/delegate. The investigator/delegate and the subject will sign the IPMP at the screening visit and a copy of the IPMP will be given to the subject.

A sample of the IPMP template is provided as Appendix 1.

Depending on the background pain medication(s) of the subject, and provided that the medication is not categorized as forbidden concomitant medication [Section 5.2.4], the rescue pain medication(s) may include one or more of the following options:

- Initiation or dose escalation of adjuvant pain medication(s) (i.e., anti-epileptics, TCAs, SNRIs, SSRIs);
- Initiation or dose escalation of opioid analgesic drugs;

- Initiation or dose escalation of non-opioid analgesics drugs (i.e., non-steroidal anti-inflammatory drugs [NSAIDs], topical analgesics).

The subject will be prescribed the rescue pain medication(s) listed in his/her IPMP. In the event of pain worsening that is not bearable, the subject will be instructed to:

- Apply the IPMP agreed with his/her investigator/delegate at the time of the screening visit;
- Contact his/her investigator/delegate as soon as possible.

The investigator/delegate will advise the subject on pain treatment adjustment in the event of unbearable pain and will document the discussion in the hospital charts.

If, after the modification of pain treatment, the subject still suffers from unbearable pain, the investigator/delegate should discuss alternative therapeutic options with the subject, including the possibility to (re-)initiate ERT treatment (if available at the site).

Details on the pain medications taken for unbearable pain will be collected in the eDiary [see Section 5.2.5.2].

5.2.3.3 Antidepressants used to treat depression

Subject must not have initiated antidepressants (i.e., SNRIs, SSRIs, TCAs) within 4 weeks prior to screening. The doses of antidepressants used by the subject at screening must be stable for at least 4 weeks prior to screening.

During the study, initiation or dose adjustment are allowed based on investigator judgment.

5.2.4 Forbidden concomitant therapy

5.2.4.1 Enzyme replacement therapy (agalsidase alfa, agalsidase beta)

Subjects on ERT with neuropathic pain at screening ("switch") must agree to stop ERT administration for about 8 months (6–7 weeks screening + 6 months of double-blind treatment). Recently published recommendations by the European Fabry Working Group have defined ERT stopping criteria which include the lack of response for 12 months when the sole "indication" for ERT is neuropathic pain, and the subject's request to stop ERT [Biegstraaten 2015].

Subjects off ERT with neuropathic pain at screening ("naïve" or "pseudo-naïve") who are planned for imminent initiation of treatment with ERT are not eligible for enrollment in the study [see Section 4.4], and initiation of ERT will therefore not be withheld for these subjects.

Although discouraged throughout the whole study, subjects may be allowed to (re-)initiate ERT treatment (if available at that site) if judged medically required due to significant FD

progression (e.g., significant renal function deterioration or cardiac function deterioration) based on investigator judgment. In such case, the subject may continue taking the study treatment.

5.2.4.2 Inhibitors of glucosyl ceramide synthase, α-galactosidase A chaperone, investigational drugs

To avoid concomitant administration of medications that would either compete with lucerastat as they have a similar mechanism of action or have an uncertain effect on neuropathic pain in subjects with FD, the following concomitant therapies are forbidden from screening visit until the EOT visit or the last visit of the PTOP (as applicable):

- Any inhibitor of GCS (e.g., miglustat, eliglustat);
- Any α-GalA chaperone (e.g., migalastat);
- Any other investigational drug (e.g., ibiglustat/venglustat, pegunigalsidase alfa).

If a subject takes any of the forbidden medications listed in Section 5.2.4, the investigator/delegate must contact the sponsor to discuss further follow-up actions including stopping/interrupting study treatment as appropriate.

5.2.5 Reporting of previous / concomitant therapy in the electronic case report form and electronic diary

5.2.5.1 Fabry disease-specific therapies

Any previous administration (at any time prior to screening) of ERT (i.e., agalsidase alfa [Replagal®], agalsidase beta [Fabrazyme®]) will be recorded in the eCRF.

For treatments received in the last 12 months prior to screening, start/end dates of administration, reason for discontinuation and dose regimen if known, will be recorded in the eCRF.

For treatments stopped before the last 12 months prior to screening, start/end dates, dose regimen, and reason for discontinuation if available in the hospital chart will be reported in the eCRF.

In addition, history of ERT infusion reactions including anaphylactic and allergic reactions will be collected in the eCRF.

ERT infusion reactions [Fabrazyme® USPI, Replagal® SmPC] include:

- Life-threatening anaphylactic and severe allergic reactions;
- Localized angioedema (including swelling of the face, mouth, and throat);
- Bronchospasm;

- Generalized urticaria, rash, pruritus, flushing;
- Dysphagia;
- Hypotension, hypertension;
- Tachycardia, bradycardia;
- Dyspnea, chest discomfort, chest pain, peripheral edema;
- Nasal congestion, throat tightness;
- Nausea, vomiting, abdominal pain, diarrhea;
- Paresthesia, pain in extremity, myalgia;
- Pyrexia, feeling hot or cold, chills, sweating, hives;
- Headache, dizziness/lightheadedness;
- Fatigue, somnolence.

If ERT therapy is (re-)initiated during the course of the study [see Section 5.2.4.1], the date of each ERT infusion, dose and detailed reason for (re-)initiation will be recorded in the eCRF (e.g., treatment-emergent renal or cardiac signs and symptoms fulfilling class I criteria recommendation to initiate ERT [Biegstraaten 2015]).

Other therapies (experimental or not) used to treat FD (e.g., inhibitor of GCS [e.g., miglustat, eliglustat, ibiglustat/venglustat], α-GalA chaperone [e.g., migalastat], or ERT [e.g., pegunigalsidase alfa / PRX-102, moss-aGal]) in the past 12 months will be recorded in the eCRF. For each therapy, the start/end dates, route of administration, and reason for discontinuation will be recorded. In addition, dose and frequency of administration should be recorded if available.

5.2.5.2 Pain medications

Previous pain medications [see Section 5.2.3.2 for definitions] if discontinued less than 30 days prior to signing the ICF and pain medication ongoing at the screening visit must be recorded in the eCRF. The generic name, start dates and end dates (if applicable) of administration, route, dose regimen, and indication will be recorded in the eCRF.

Starting at the screening visit, the subject will be asked on a daily basis to record the use of pain medication (e.g., dose regimen, initiation of a new medication) in the eDiary [see Section 7.2.2.1 for more detail on the eDiary] until the EOT visit or the last visit of the PTOP (as applicable).

Pain medications taken by the subject during the FU period after he/she has returned the eDiary will be collected in the eCRF.

5.2.5.3 Other therapies

The use of other (i.e., not covered in Sections 5.2.5.1 and 5.2.5.2) study-concomitant therapy (including contraceptives and traditional and alternative medicines, e.g., plant-, animal-, or mineral-based medicines) will be recorded in the eCRF.

Any other previous therapy must be recorded in the eCRF if discontinued less than 30 days prior to signing of the ICF.

The generic name, start/end dates of administration, route, dose regimen, and indication will be recorded in the eCRF.

6 STUDY ENDPOINTS

6.1 Efficacy endpoints

6.1.1 Primary efficacy endpoint

The primary efficacy endpoint is:

- Change from baseline to Month 6 in the "modified" BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours".

This endpoint is determined from subject daily scoring of neuropathic pain intensity in an eDiary [see Section 7.2.2.1].

The rules used to derive the baseline and Month 6 modified BPI-SF3 scores are described in Section 10.2.1.

6.1.2 Secondary efficacy endpoints

The secondary efficacy endpoints are:

- Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline.

This endpoint is determined from subject daily scoring of abdominal pain intensity in an eDiary [see Section 7.2.2.1].

The rules used to derive the baseline and Month 6 average abdominal pain NRS-11 scores are described in Section 10.2.2.1.

The definition of a subject considered to have GI symptoms at baseline is provided in Section 10.1.4.

- Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale (BSS) consistency Type 6 or 7 in subjects with GI symptoms at baseline.

This endpoint is determined from subject's daily reporting of bowel movement and stool consistency scoring in an eDiary [see Section 7.2.2.1].

The rules used to derive the baseline and Month 6 number of days with at least 1 stool with BSS Type 6 or 7 are described in Section 10.2.2.2.

The definition of a subject considered to have GI symptoms at baseline is provided in Section 10.1.4.

- Change from baseline to Month 6 in plasma Gb3.

6.1.3 Efficacy estimands

The estimands targeted by the primary and secondary efficacy objectives and analyses are defined in Table 3.

A broad treatment policy strategy will be applied for all estimands defined in Table 3, i.e., all collected endpoint data are used in the analyses regardless of occurrence of any intercurrent events (ICEs). Expected ICEs are premature treatment discontinuation as well as use of other medications.

FD medications (e.g., ERT) are forbidden by the protocol as they are not part of the treatment condition of interest [see definition in Table 3]. However, considering that forbidden medications could be taken in clinical practice, data collected during use of forbidden medications will be included in the analyses.

Table 3 Estimands for the primary and secondary efficacy objectives and analyses

Estimand	Target population	Treatment condition of interest	Endpoint	Strategy for addressing intercurrent events	Population-level summary
Primary Estimand	Adult subjects with FD as defined by the inclusion and exclusion criteria in section 4.3 and 4.4	Lucerastat (dose ranging from 250 mg to 1000 mg b.i.d. based on subject's eGFR value) as monotherapy which will be compared to placebo.	Change from baseline to Month 6 in the modified BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours".	Treatment policy, i.e., all collected endpoint data are used regardless of premature treatment discontinuation or use of other medication (e.g., pain medication, ERT).	Mean change from baseline to Month 6, summarized as the difference between lucerastat and placebo.
Secondary Estimand #1	Adult subjects with FD as defined by the inclusion and exclusion criteria in section 4.3 and 4.4.	Lucerastat (dose ranging from 250 mg to 1000 mg b.i.d. based on subject's eGFR value) as monotherapy which will be compared to placebo.	Change from baseline to Month 6 in plasma Gb3.	Treatment policy, i.e., all collected endpoint data are used regardless of premature treatment discontinuation or use of other medication (e.g., pain medication, ERT).	Mean change from baseline to Month 6, summarized as the difference between lucerastat and placebo.
Secondary Estimand #2	Adult subjects with FD as defined by the inclusion and exclusion criteria in section 4.3 and 4.4 with GI symptoms at baseline.	Lucerastat (dose ranging from 250 mg to 1000 mg b.i.d. based on subject's eGFR value) as monotherapy which will be compared to placebo.	Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours".	Treatment policy, i.e., all collected endpoint data are used regardless of premature treatment discontinuation or use of other medication (e.g., pain medication, ERT, GI symptomatic treatments).	Mean change from baseline to Month 6, summarized as the difference between lucerastat and placebo.
Secondary Estimand #3	Adult subjects with FD as defined by the inclusion and exclusion criteria in section 4.3 and 4.4 with GI symptoms at baseline.	Lucerastat (dose ranging from 250 mg to 1000 mg b.i.d. based on subject's eGFR value) as monotherapy which will be compared to placebo.	Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale consistency Type 6 or 7.	Treatment policy, i.e., all collected endpoint data are used regardless of premature treatment discontinuation or use of other medication (e.g., medication used to treat/prevent diarrhea).	Win ratio of change from baseline to Month 6 between lucerastat and placebo.

b.i.d. = twice daily; BPI-SF3 = Brief Pain Inventory – Short Form item 3; eGFR = estimated glomerular filtration rate; ERT = enzyme replacement therapy; FD = Fabry disease; Gb3 = globotriaosylceramide; GI = gastrointestinal; NRS-11 = 11-point numerical rating scale.

6.1.4 Other efficacy endpoints

6.1.4.1 Renal function endpoints

- Subject eGFR slope from baseline to Month 6;
- Changes from baseline to Month 6 in urine albumin-to-creatinine ratio (UACR).

Of note, long-term outcome of lucerastat on renal function parameters will be further assessed in the OLE study (separate protocol).

6.1.4.2 Echocardiography-based endpoints

- Change from baseline to Month 6 in LVMI, posterior wall thickness, left ventricular mean wall thickness, left ventricular ejection fraction (LVEF), left ventricular end diastolic and end systolic volumes, left atrial volume, as measured by echocardiography.

Of note, long-term outcome of lucerastat on echocardiography parameters will be further assessed in the OLE study (separate protocol).

6.1.4.3 Pain medication endpoints based on daily entries in electronic diary

- Subject mean weekly dose of opioid analgesics from baseline up to Month 6.

  Doses of opioids will be converted to equianalgesic dose of oral morphine;

- Use of significant rescue pain therapy from baseline up to Month 6.

  Significant rescue pain therapy is defined as any initiation or dose escalation of anti-epileptics, TCAs, SNRIs/SSRIs, or opioid analgesic drugs as recorded in the eDiary;

- Total number of days on significant rescue pain therapy from baseline up to Month 6.

6.1.4.4 Clinical symptoms endpoints based on data collected at site visits

- Change from baseline to Month 6 in the subject's rating of item 5 score of the BPI-SF ("pain on the average in the last 24 hours");
- Change from baseline to Month 6 in the total score of the subject's rating of item 9 of the BPI-SF (7 pain interference questions: "general activity", "mood", "walking ability", "normal work", "relation with other people", "sleep", "enjoyment of life");
- Change from baseline to Month 6 in the subject's rating of severity of neuropathic pain severity as measured by the Patient Global Impression of Severity of neuropathic Pain (PGIS-P);

- Subject rating of change in the overall severity of neuropathic pain since study treatment start as measured by the Patient Global Impression of Change (PGIC) in neuropathic Pain Severity (PGIC-PS) at Month 6;
- Change from baseline to Month 6 in the subject's rating of disease severity as measured by the Patient Global Impression of Severity of Disease (PGIS-D);
- Subject rating of change in disease severity since study treatment start as measured by the PGIC in Disease Severity (PGIC-DS) at Month 6;
- Change from baseline to Month 6 in the total score of the subject's rating of the Center for Epidemiologic Studies Depression Scale Revised (CESD-R-20).

6.1.4.5 Treatment failure

- Time to treatment failure from baseline up to Month 6.

Treatment failure is defined as:

- Initiation or re-initiation of ERT; or
- Permanent study treatment discontinuation for any reason.

6.1.5 Overview of efficacy endpoints

Table 4 provides a list of the study efficacy endpoints.

Table 4 Type of efficacy endpoints

Primary endpoint	Change from baseline to Month 6 in the "modified" BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours".
Secondary endpoints	Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline. Change from baseline to Month 6 in the number of days with at least one stool of a BSS consistency Type 6 or 7 in subjects with GI symptoms at baseline. Change from baseline to Month 6 in plasma Gb3.
Other efficacy endpoints	
Renal function endpoints	Subject eGFR slope from baseline to Month 6. Change from baseline to Month 6 in UACR.
Echocardiography-based endpoints	Change from baseline to Month 6 in LVMI, posterior wall thickness, left ventricular mean wall thickness, LVEF, left ventricular end diastolic and end systolic volumes, left atrial volume as measured by echocardiography.
Pain medication endpoints based on daily entries in eDiary	Subject mean weekly dose of opioid analgesics from baseline up to Month 6. Use of significant rescue pain therapy from baseline up to Month 6. Total number of days on significant rescue pain therapy from baseline up to Month 6.
Clinical symptoms endpoints based on data collected at site visits	Change from baseline to Month 6 in the subject's rating of item 5 score of the BPI-SF ("pain on the average in the last 24 hours"). Change from baseline to Month 6 in the total score of the subject's rating of item 9 of the BPI-SF (7 pain interference questions: "general activity", "mood", "walking ability", "normal work", "relation with other people", "sleep", "enjoyment of life"). Change from baseline to Month 6 in the subject's rating of severity of neuropathic pain severity as measured by the PGIS-P. Subject rating of change in the overall severity of neuropathic pain since study treatment start as measured by the PGIC-PS at Month 6.

	Change from baseline to Month 6 in the subject's rating of disease severity as measured by the PGIS-D. Subject rating of change in disease severity since study treatment start as measured by the PGIC-DS at Month 6. Change from baseline to Month 6 in the total score of the subject's rating of the CESD-R-20.
Treatment failure	Time to treatment failure from baseline up to Month 6.

BSS = Bristol Stool Scale; BPI-SF3/5/9 = Brief Pain Inventory - Short Form item 3/5/9; CESD-R-20 = Center for Epidemiologic Studies Depression Scale Revised; eGFR = estimated glomerular filtration rate; Gb3 = globotriaosylceramide; GI = gastrointestinal; LVEF = left ventricular ejection fraction; LVMI = left ventricular mass index; NRS-11 = 11-point numerical rating scale; PGIC-DS = Patient Global Impression of Change in Disease Severity; PGIS-D = Patient Global Impression of Severity of Disease; PGIC-PS = Patient Global Impression of Change in neuropathic Pain Severity; PGIS-P = Patient Global Impression of Severity of neuropathic Pain; UACR = urine albumin-to-creatinine ratio.

6.2 Safety endpoints

- Treatment-emergent AEs and SAEs;
- AEs leading to premature discontinuation of study treatment;
- Change from baseline to each visit up to Month 6 in vital signs.

  Vital signs include systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), and body weight;
- Treatment-emergent marked[a] abnormalities for vital signs up to Month 6;
- Change from baseline to each visit up to Month 6 and from pre-dose to 2 hours and 4 hours post dose at Month 1 in 12-lead ECG variables.

  ECG variables include HR, PR, QRS, QT, corrected QT (QTc) and morphological abnormalities as defined by the ECG provider;
- Treatment-emergent marked[a] abnormalities for quantitative 12-lead ECG variables up to Month 6;
- Change from baseline to each visit up to Month 6 in laboratory variables [see list in Section 7.2.4.2];
- Treatment-emergent marked[a] abnormalities for selected laboratory variables up to Month 6.

[a] The selection of marked abnormalities considered for the analyses will be based on standard definitions and described in the SAP.

6.3 Quality of life endpoints

- Change from baseline up to Month 6 in subject rating of the 36-Item Short Form Health Survey Version 2 (SF-36v2™) domain and component scores.

6.4 Pharmacokinetic endpoints

- Trough plasma concentration of lucerastat at each visit up to Month 6.

6.4.1 Pharmacokinetic profile sub-study

The plasma PK parameters of lucerastat will be derived by non-compartmental analysis of the plasma concentration-time data from PK samples collected at Month 1 in a subset of subjects:

- AUC during one dosing interval (AUC_τ) in a subset of subjects;
- C_{max} during one dosing interval in a subset of subjects;
- t_{max} during one dosing interval;
- The terminal disposition rate constant (λ_Z) and the $t_{½}$.

The measured individual plasma concentrations of lucerastat will be used to directly obtain C_{max} and t_{max}. AUC_τ will be calculated according to the linear trapezoidal rule using the measured concentration-time values above the limit of quantification during one dosing interval. The PK parameters will be calculated based on the actual blood sampling time points.

For mean value calculations, all values below the limit of quantification (BLQ) will be set to zero. If > 50% of the values at a given time point are BLQ, no mean value will be calculated. Mean concentration-time profiles will be generated using these criteria.

AUC_τ, C_{max}, and $t_{½}$ are assumed to be log-normally distributed [Julious 2000].

If necessary, blood samples collected for determination of lucerastat plasma concentrations will be used for identification of possible metabolites. Results of metabolite investigations will be reported separately from the Clinical Study Report (CSR).

6.5 Biomarker endpoints

- Change from baseline to each visit up to Month 6 in plasma Gb3, lysoGb3 and their metabolic precursors (GlcCer and LacCer);
- Change from baseline to each visit up to Month 6 in urinary Gb3 and lysoGb3.

6.6 Rationale for primary and secondary efficacy endpoints

6.6.1 Rationale for evaluating neuropathic pain at Month 6

Neuropathic pain is a major symptom of subjects with FD, being one of the earliest, most common, and most disabling clinical manifestations, impacting QoL of both male and female subjects with FD [Gold 2002, Hoffmann 2005, Eng 2007]. Subjects may experience permanent pain of variable intensity as well as pain attacks or crises [Biegstraaten 2012, Politei 2016, Üceyler 2014]. In the Fabry Registry, neuropathic pain has been reported in 62% of male and 41% of female subjects [Eng 2007]. Subjects with FD with neuropathic pain have also a higher risk of developing clinical complications (kidney, heart, hypoacousia) and depression [Gold 2002, Cole 2007, Bolsover 2014, Kaminsky 2014]. Neuropathic pain is not adequately managed by conventional pain medications or by ERT [see Section 1.1.2].

Demonstration of a clinically meaningful improvement in neuropathic pain symptom to establish drug effectiveness has been acknowledged by the FDA in their draft guidance on development of drugs for FD treatment [FDA 2019]. In the absence of regulatory guidelines specific to the development of treatment for FD at the time of the first version of the protocol, the methodology used in the study to assess the effect of lucerastat on neuropathic pain has been based whenever applicable on the CHMP guideline for neuropathic pain [CHMP 2007], and FDA guidance on chronic pain [FDA 2014], and PROs [FDA 2009].

While both the FDA and the CHMP recommend at least a 3-month duration for studies evaluating analgesia in a chronic pain condition [FDA 2014, CHMP 2017], it is postulated that the potential effects of lucerastat on the established clinical symptoms of neuropathic pain would already be measurable at Month 6 [see Section 3.2].

Pain intensity has been reported to be higher during pain attacks and crises as compared to chronic pain [Üceyler 2014]. The frequency of pain attacks and crises may vary from daily occurrence to once a year. About 30–40% of subjects with FD who have neuropathic pain experience pain attacks or pain crises 1–4 times a month [Üceyler 2014]. Therefore, the baseline and Month 6 values will be derived from the subject's neuropathic pain data collected over a period of 4 weeks [see Section 10.2.1], i.e., a time duration reasonably long enough to capture a true reflection of the subject's score accounting for the variability of neuropathic pain intensity in subjects with FD.

Published guidelines on neuropathic pain [CHMP 2007] and chronic pain [FDA 2014] recommend assessing the efficacy of treatment on pain preferably using uni-dimensional pain scales (e.g., NRS-11) as primary outcome.

The BPI-SF is a frequently used, self-administered questionnaire, developed to assess the severity and impact of pain [Cleeland 2002]. The BPI-SF has become one of the most

widely used measurement tools for assessing clinical pain and is one of the recommended regular assessments to be performed in the two global FD registries [Hernberg-Ståhl 2006, fabrydisease.org]. The 24-hour recall period of the BPI-SF items is considered an appropriate time period for assessing pain intensity in line with the FDA and CHMP guidelines [CHMP 2017, FDA 2014].

In clinical studies, the BPI-SF item "pain at its worst in the last 24 hours" (BPI-SF3) has been used to assess pain severity and its use is supported by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) recommendations for assessing pain in clinical trials [Dworkin 2005]. While the BPI-SF3 is a well-documented instrument to assess pain in general, it is not specific to neuropathic pain in FD. Therefore, a "modified" BPI-SF3 including a definition of neuropathic pain will be used in this study [see Section 7.2.2.2.1].

As per CHMP guideline on pain [CHMP 2017], the weekly averages of the daily measurement compared to baseline are commonly used as the primary efficacy variable in long term studies. The responder analysis should be provided in addition to the primary efficacy analyses with 30% or 50% reduction in pain intensity compared to baseline for chronic pain conditions. The benchmark commonly used to define the clinical relevance of changes in pain intensity is based on the clinically important differences in 11-point pain intensity scales following the recommendation of the IMMPACT consensus on the clinical significance of change across pain states and the CHMP guideline on neuropathic pain [Farrar 2001, CHMP 2007, Dworkin 2008, Farrar 2010]. In 15 chronic pain (including diabetic peripheral neuropathy) clinical trials, a decrease of 30% in pain intensity scale was associated with the categories "much improved" or "very much improved" in a 7-point PGIC regardless of the underlying disease, treatment administered and subject's age or sex [Farrar 2001, Farrar 2010].

6.6.2 Rationale for evaluating gastrointestinal symptoms (abdominal pain, diarrhea) at Month 6

GI involvement is one of the earliest clinical manifestations and the second most common sign after neuropathic pain reported in childhood [Eng 2007, Hoffmann 2007a]. GI symptoms were reported in up to 52% of adult subjects with FD [Hoffmann 2007a]. GI symptoms in FD are somewhat similar to those associated with functional disorders such as IBS [Keshav 2006]. Abdominal pain and diarrhea are the most frequent GI symptoms, affecting 32% and 20% respectively of adult patients with FD, while constipation has been reported in 13% of patients [Hoffmann 2007a]. Gender differences have been reported in the FOS with diarrhea being more frequently reported by male (25.9%) than female (16.7%) patients and constipation more frequently reported by female (16.7%) than male (8.6%) patients [Hoffmann 2007a]. GI symptoms are not adequately managed by ERT [see Section 1.1.2].

In the absence of regulatory guidelines specific to the development of treatment for FD at the time of the first version of the protocol, the methodology used in the study to assess the effect of lucerastat on GI symptoms has been based on the IBS [FDA 2012] and PRO [FDA 2009] guidance. According to the draft FDA guidance on development of drugs for FD treatment [FDA 2019], drug effectiveness can be established with the demonstration of clinically meaningful improvement in GI symptoms (abdominal pain, diarrhea, constipation).

Similarly to the neuropathic pain symptom, it is postulated that the potential effects of lucerastat on GI symptoms (abdominal pain and diarrhea) result from the reduction of Gb3 and lysoGb3 storage in the GI tract [see Section 1.3]. As for neuropathic pain, it is expected that the effect of lucerastat on GI symptoms would already be measurable at Month 6 in those subjects who had the corresponding symptom at baseline. Similar to neuropathic pain, GI symptoms may vary in frequency and intensity. Some subjects with FD describe a cyclic pattern of alternating diarrhea and constipation interspersed with periods of quiescence and normal bowel movements, making diagnosis and management particularly difficult [Keshav 2006]. Therefore, the baseline and Month 6 values will be derived from the subject's abdominal pain and diarrhea data collected over a period of 4 weeks [see Sections 10.2.2.1 and 10.2.2.2], i.e., a time duration reasonably long enough to capture a true reflection of the subject's score accounting for the variability of diarrhea and abdominal pain symptoms in subjects with FD.

Published IBS guidelines [FDA 2012, CHMP 2014] recommend assessing the efficacy of treatment on abdominal pain intensity in subjects with moderate to severe abdominal pain by using uni-dimensional pain scales (e.g., NRS-11) that ask subjects to rate their abdominal pain at its worst in the last 24 hours on a daily basis. This is also in line with the recommendation from the IMMPACT consensus conference [Dworkin 2005]. The criteria used to define a subject with moderate to severe abdominal pain at baseline are consistent with the published IBS guidelines [FDA 2012]. The abdominal pain intensity rating scale used in this study [see Section 7.2.2.2.2] has been previously tested in a cognitive debriefing / usability testing study conducted in subjects with FD.

Published IBS guidelines [FDA 2012] recommend assessing the efficacy of treatment on diarrhea in subjects with diarrhea by using the BSS to assess the number and consistency of the stools on a daily basis. The BSS provides a visual and verbal description of stool consistency and form and is considered an appropriate instrument for capturing stool consistency in IBS trials [Lewis 1997]. The criteria used to define a subject with diarrhea at baseline are consistent with the published IBS guidelines [FDA 2012]. The questions used to assess the number and consistency of the stools in this study [see Section 7.2.2.2.3] have been previously tested in a cognitive debriefing / usability testing study conducted in subjects with FD.

6.6.3 Rationale for evaluating plasma levels of globotriaosylceramide at Month 6

Changes in plasma levels of Gb3 will be evaluated in this study as a marker of the PD effect of SRT with lucerastat in subjects with FD.

In the exploratory Phase 1b study in subjects with FD receiving ERT, lucerastat treatment was associated with a fast, marked and consistent reduction in plasma Gb3 concentration [see Section 1.2.3 and Lucerastat IB]. In this study, it is expected that plasma Gb3 levels will be reduced in the lucerastat arm compared to the placebo arm after 6 months, in both ERT "treatment-naïve" / "pseudo-naïve" subjects and "switch" subjects.

Gb3 concentrations are highly elevated in plasma, urine and target organs of subjects with FD as a direct result of the reduced or absent α-GalA activity caused by mutations of the *GLA* gene. In subjects with FD, ERT gives rise to a 50% reduction of plasma Gb3 relative to baseline after 3–6 months of treatment, and no further reduction after longer periods of treatment [Bekri 2006].

Plasma lysoGb3, which probably originates from Gb3, is also elevated in subjects with FD, but is not favored as a PD marker for this study due to the smaller FD induced absolute increase than Gb3 [Van Breemen 2011]. In subjects with FD, the reduction in plasma lysoGb3 achieved with ERT in subjects with FD is quantitatively much lower than that achieved in plasma Gb3 [Van Breemen 2011].

7 VISIT SCHEDULE AND STUDY ASSESSMENTS

7.1 Study visits

The study visits and their respective time windows are listed in Table 5 and Table 6.

In the event of premature discontinuation of study treatment, the EOT visit must take place as soon as possible and no later than 7 days after the last dose of study treatment. In such case, all assessments planned at the Month 6 visit must be performed at the EOT visit. Subjects who prematurely discontinue study treatment for any reason will not be replaced.

An exit interview will be conducted by telephone as soon as possible after the end of the study treatment phase (or EOT visit), ideally within 2 weeks in English-speaking subjects at US and Canadian sites.

Safety follow-up information will be collected during the FU1 visit (all subjects) and FU2 visit (only in fertile males) via telephone call. At the FU1 visit, safety follow-up information includes use of concomitant therapy, AE/SAEs, result of pregnancy test, and reporting of pregnancy (as needed) in female subjects or male subjects' female partners. Fertile male subjects will be reminded to continue using contraception until at least 3 months following EOT. At the FU2 visit, safety follow-up information includes reporting of pregnancy (as needed) in male subjects' female partners.

For subjects followed in the PTOP, safety follow-up information may also be collected during a site visit if it coincides with the originally scheduled visits.

7.1.1 Screening/re-screening

7.1.1.1 Screening

The date of the screening visit is the date when the ICF is signed [see Section 12.3 for informed consent procedure].

The subjects who agree to participate in the study and the investigator/delegate must sign the ICF prior to any study-related assessment or procedure.

If the signing of ICF and performance of the first study-specific procedures or assessments take place on the same day, it must be clear from the source documents that informed consent was obtained prior to any study-specific procedures being performed. If a study-specific procedure or assessment has been performed as part of routine assessments on the day of the screening visit and the results are available prior to the subject signing the ICF, such procedure or assessment may be used and does not have to be repeated (e.g., vital signs). In such cases, it must be clear from the source documents when and for which reason the assessment was done prior to the signing of the ICF.

For convenience reasons, study-specific procedures or assessments can take place on different days during the screening period.

After the ICF has been signed, the investigator/delegate contacts the IRT system to get a subject number allocated to the subject.

Subjects who have signed the ICF when the enrollment target has been met may still be randomized.

7.1.1.2 Re-screening

Subjects who did not meet the criteria for participation in the study (i.e., screen failure) may be re-screened once if the reason for non-eligibility was transient (e.g., abnormal laboratory test, insufficient wash-out period of a forbidden medication, insufficient time window for allowed medications or previous medical events, having received ERT in the last 6 months). If the reason for non-eligibility is the absence of moderate to severe neuropathic pain (i.e., inclusion criteria #9 not met), re-screening is permitted only after 1 month.

A new ICF must be signed prior to re-screening the subject if more than 3 months have elapsed since the first ICF signature. Re-screened subjects will be assigned the same subject number as for the initial screening.

7.1.2 Unscheduled visits

Unscheduled visits may be performed at any time during the study and will be recorded in the eCRF. Physical examination, vital signs, ECG and laboratory assessments must be performed at each unscheduled visit. PK and biomarker sampling should not be performed during unscheduled visits. Other assessments are performed at the discretion of the investigator.

After an unscheduled visit, the regular scheduled study visits must continue according to the planned visit and assessment schedule.

Table 5 Visit and assessment schedule: double-blind treatment period

PERIODS	Name	SCREENING	TREATMENT PERIOD						FOLLOW-UP PERIOD		Unscheduled
	Duration	6 to 7 weeks	6 months					Up to 3 months		NA	
VISITS	Number	1	2	3	4	5	6	7	8	9	10	U1
	Name	Screening	Randomization	Month 1	Month 2 (phone)	Month 3	Month 4 (phone)	Month 5	Month 6 or EOT[a]	FU1[b]	FU2[b]	Unscheduled
	Time window	Day −49 [max] / Day −42 [min] to Day −1	Day 1	Day 21 to Day 38	Day 54 to Day 68	Day 84 to Day 98	Day 115 to Day 129	Day 145 to Day 159	Day 169 to Day 197	30 to 37 days after last study treatment dose	91 to 105 days after last study treatment dose	Any day between ICF signature and EOS
Informed consent		X										
Eligibility		X[c]	X									
Demographics		X										
FD history and treatment		X										
Medical history		X										
Previous & concomitant therapy		X	X	X	X	X	X	X	X	X		*(X)*
IPMP[d]		X	X	X	X	X	X	X	X			*(X)*
eDiary training/retraining		X	X	X	X	X	X	X	X[a]			
Pain medication use **		← X (collected daily in eDiary)[a] →								X[e]		
Physical examination		X	X	X		X		X	X			X
Vital signs (BP, HR, body weight)		X (+ height)	X	X		X		X	X			X
12-lead ECG (trough)*		X	X	X[f]		X		X	X			X
Echocardiography*			X						X			*(X)*
Laboratory tests (urine, blood) fasted state *		X (+stool[g])	X[h]	X		X		X	X			X

Pregnancy test[i]	X	X	X	X	X	X	X	X	X		*(X)*
Biomarker sampling (urine/plasma at trough)*	X-	X	X		X		X	X			
Research biomarker sampling (urine/plasma/serum at trough)*		X						X			
PK sampling (trough)*			X		X		X	X			
PK profile sampling (sub-study)*			X[j]								
Neuropathic pain NRS-11 (modified BPI-SF3)**	← X (collected daily in eDiary)[a] →										
Abdominal pain NRS-11**	← X (collected daily in eDiary)[a] →										
Stool number& consistency **	← X (collected daily in eDiary)[a] →										
BPI-SF (Complete)**		X	X		X		X	X			
PGIS-D, PGIS-P, PGIC-DS & PGIC-PS**		X (only PGIS-D & PGIS-P)	X		X		X	X			
SF-36**		X						X			
CESD-R-20**		X						X			
Exit interview (sub-study)*[k]								X			
SD dispensing/return		X	X		X		X	X			*(X)*
SAEs/AEs	X	X	X	X	X	X	X	X	X		X
Pregnancy report***	X	X	X	X	X	X	X	X	X	X[l]	X

(X) = optional. * collected by vendor. ** collected in eDiary. *** only reported to sponsor Global Drug Safety.

[a] After completion of the Month 6 visit, those subjects will be proposed to enroll in a separate OLE study. Subjects who prematurely discontinue SD prior to the Month 6 visit should have an EOT visit scheduled not later than 7 days after the last dose of SD. In such case, all assessments planned at the Month 6 visit must be performed at the EOT visit. Those subjects will also have a safety follow-up and will enter into the PTOP [see Table 6]. eDiary retraining is not required at EOT if subject does not enter into the PTOP. The subject will return the eDiary to the site personnel at the EOT visit or at the last PTOP visit (whichever is last).

[b] Visit(s) performed by telephone by the investigator only for subjects who do not enter the separate OLE study. For those subjects, the EOS corresponds to the FU1 visit, FU2 visit or to the last visit of the PTOP, whichever is last.

[c] If the *GLA* gene mutation is not known (i.e., male subject diagnosed based on residual α-GalA activity), the gene mutation will be determined by the site locally at the screening visit.
[d] IPMP will be applicable starting at screening visit and until EOT visit or the last visit of the PTOP (as applicable).
[e] Pain medications taken by the subject during the FU period after he/she has returned the eDiary will be collected in the eCRF.
[f] At the Month 1 visit, 2 additional ECGs will be performed 2 and 4 hours after SD administration.
[g] Stool samples will be collected only in subjects presenting diarrhea symptoms at the screening visit.
[h] α-GalA activity will be measured at the screening and randomization visits only.
[i] Serum pregnancy test at screening visit. Urinary pregnancy tests at all other visits and performed at home on a monthly basis between the visits until the FU1 visit. Result of the pregnancy tests are not collected in the eCRF.
[j] Subset of subjects: blood samples to be drawn prior to study treatment administration (pre-dose), and 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 6 h, 8 h, 10 h, and 12 h thereafter.
[k] Exit phone interviews conducted in US and Canadian subjects ideally within 2 weeks after the Month 6 or EOT visit.
[l] Only pregnancy of female partner of male subjects.

α-GalA = α-galactosidase A; AE = adverse event; BP = blood pressure; BPI-SF = Brief Pain Inventory-Short Form; CESD-R-20 = Center for Epidemiologic Studies Depression Scale Revised; ECG = electrocardiogram; eCRF = electronic case report form; EOS = End-of-Study; EOT = End-of-Treatment; FD = Fabry disease; FU = follow-up; GI = gastrointestinal; HR = heart rate; ICF = informed consent form; IPMP = Individual Pain Management Plan; NRS-11 = 11-point numerical rating scale; OLE = open-label extension; PGIC-DS = Patient Global Impression of Change in Disease Severity; PGIC-PS = Patient Global Impression of Change in Pain Severity; PGIS-D = Patient Global Impression of Severity of Disease; PGIS-P= Patient Global Impression of Severity of neuropathic Pain; PK = pharmacokinetic(s); PTOP = post-treatment observation period; SAE = serious adverse event; SD = study drug; SF-36 = Short Form Health Survey.

Table 6 Visit and assessment schedule: post-treatment observation period

VISITS	Number	3PTOP	4PTOP	5PTOP	6PTOP	7PTOP	8PTOP
	Name	Month 1PTOP	Month 2PTOP (phone)	Month 3PTOP	Month 4PTOP (phone)	Month 5PTOP	Month 6PTOP or End of PTOP
	Time window	Day 21 to Day 38	Day 54 to Day 68	Day 84 to Day 98	Day 115 to Day 129	Day 145 to Day 159	Day 169 to Day 197
Concomitant therapy		X	X	X	X	X	X
IPMP[a]		X	X	X	X	X	X
eDiary training/retraining		X	X	X	X	X	
Pain medication use **		← X (collected daily in eDiary)[b] →					
Physical examination		X		X		X	X
Vital signs (BP, HR, body weight)		X		X		X	X
12-lead ECG*		X		X		X	X
Echocardiography*							X
Laboratory tests (urine, blood) fasted state *		X		X		X	X
Biomarker sampling (urine/plasma)*		X		X		X	X
Research biomarker sampling (urine/plasma/serum)*							X
Neuropathic pain NRS-11 (modified BPI-SF3)**		← X (collected daily in eDiary)[b] →					
Abdominal pain NRS-11**		← X (collected daily in eDiary)[b] →					
Stool number& consistency **		← X (collected daily in eDiary)[b] →					
BPI-SF (Complete)**		X		X		X	X
PGIS-D, PGIS-P, PGIC-DS & PGIC-PS**		X		X		X	X
SF-36**							X
CESD-R-20**							X
SAEs/AEs		X	X	X	X	X	X

*collected by vendor. ** collected in eDiary.
[a] IPMP will be provided to the subject at the screening visit and will be applicable until Month 6 (or last PTOP) visit.
[b] The subject will return the eDiary to the site personnel at the EOT visit or at the last PTOP visit (whichever is last).

AE = adverse event; BP = blood pressure; BPI-SF = Brief Pain Inventory-Short Form; CESD-R-20 = Center for Epidemiologic Studies Depression Scale Revised; ECG = electrocardiogram; EOT = End-of-Treatment; HR = heart rate; IPMP = Individual Pain Management Plan; NRS-11 = 11-point numerical rating scale; PGIC-DS = Patient Global Impression of Change in Disease Severity; PGIC-PS = Patient Global Impression of Change in Pain Severity; PGIS-D = Patient Global Impression of Severity of Disease; PGIS-P= Patient Global Impression of Severity of neuropathic Pain; PTOP = post-treatment observation period; SAE = serious adverse event; SF-36 = Short Form Health Survey.

7.2 Study assessments

The study assessments are listed in Table 5 and Table 6. The assessments that are mandatory during a visit are marked with an 'X'. Optional assessments are marked with an '*(X)*'.

All study assessments performed during study visits (scheduled or unscheduled) are done by the investigator/delegate and are recorded in the eCRF, unless otherwise specified.

In exceptional circumstances, the investigator/delegate may perform some assessments shortly prior to or after the scheduled visit (with the exception of the randomization visit) as long as it is within protocol-defined visit time window.

All PROs collected on a daily basis (e.g., evening diary) or during a site visit will be recorded in an eDiary [see Section 7.2.2.1 for more details].

During site visits, subjects should complete the site visit PROs preferably at the beginning of the visit. Whenever feasible, the following order of assessments is preferred: SF-36v2™, BPI-SF, PGIS-P, PGIC-PS, PGIS-D, PGIC-DS, CESD-R-20, physical examination / vital signs, ECG, blood/urine/PK/biomarker sampling.

If the principal investigator (PI) delegates any study procedure/assessment e.g., ECG, echocardiography, blood sampling to an external facility, he/she should inform the sponsor to whom these tasks are delegated. The set-up and oversight will be agreed upon with the sponsor. The supervision of any external facilities remains the responsibility of the PI.

Calibration certificates / evidence of equipment maintenance for the below-listed equipment used to perform study assessments must be available unless the equipment is provided by the sponsor. Calibration certificates / evidence of equipment maintenance of other equipment must be available as per local requirements.

Equipment for which calibration certificates are needed:

- Temperature measurement devices for study treatment storage area and laboratory sample storage (e.g., freezer);
- ECG recorder;
- Echocardiography device. In addition, prior to the start of the study, each site must be qualified by the central echocardiography laboratory [see Section 7.2.2.7].

7.2.1 Demographics / baseline characteristics

Demographic and baseline characteristic data to be collected on all subjects include: age, sex, race, and ethnicity (if allowed in the country). Relevant medical history / current medical conditions other than those related to FD (e.g., chronic and ongoing acute

conditions, serious past conditions) present before signing of the ICF will be recorded in the eCRF. Where possible, main diagnoses and not symptoms will be recorded.

7.2.1.1 Fabry disease history

Relevant FD medical history of special interest will be captured in the eCRF and includes:

- Date and type of first known FD symptom (neuropathic pain, GI, skin, cardiac, kidney, cerebrovascular, eye, ear, bone, respiratory);
- Date of FD diagnosis;
- Diagnostic method (*GLA* gene mutation, α-GalA activity). If α-GalA was measured, sample type (e.g., plasma, leukocytes, dried blood spots), date of laboratory test, value, corresponding normal ranges and unit will be collected;
- *GLA* gene mutation, date of genetic test, and testing method used.

If the *GLA* gene mutation is not known (i.e., male subject diagnosed based on residual α-GalA activity), the gene mutation will be determined by the site locally at the screening visit.

An independent expert geneticist will be appointed by the sponsor to review and categorize in a blinded fashion the FD genetic mutations present at screening and reported by the investigator in the eCRF.

- Incident case (yes/no/not known). An incident case is defined as a subject diagnosed in the absence of known family history of FD cases;
- Neuropathic pain symptoms:
  - Date of first neuropathic pain symptom;
  - Type of neuropathic pain symptom(s): chronic pain, pain crisis/attacks, other;
  - Pain triggering factors: heat or cold, weather change, being sick or having fever, diet, stress, tiredness, physical activity, other;
  - Type of pain medication used to treat neuropathic pain in the last 12 months: anti-epileptics, TCAs, SNRIs, SSRIs, non-opioid analgesics (NSAIDs, topical analgesics), opioid analgesics.
- Other FD-related symptoms and complications:
  - Other neurological symptoms excluding neuropathic pain: heat intolerance, cold intolerance, hearing loss, tinnitus, vertigo, depression;
  - GI symptoms: diarrhea, abdominal pain / postprandial pain, abdominal discomfort, bloating, vomiting, nausea, constipation, early satiety, poor weight gain;

- Eyes: corneal verticillata, corneal opacities, corneal dystrophy, lenticular opacities, retinal vein tortuosity, conjunctival vessel disorder;
- Kidney: microalbuminuria, proteinuria, renal impairment;
- Heart: heart valve insufficiency, arrhythmias, tachycardia, bradycardia, atrial fibrillation, atrial flutter, PR interval shortened, QTc prolonged, left ventricular hypertrophy, right ventricular hypertrophy, angina pectoris, myocardial infarction, coronary artery disease, heart failure (current NYHA class should be provided);
- Respiratory: dyspnea at rest, dyspnea exertional, chronic cough, wheezing, chronic obstructive lung disease;
- Cerebrovascular: transient ischemic attacks, ischemic stroke, white matter lesions;
- Bones: osteoporosis, osteopenia;
- Skin: angiokeratoma, anhydrosis, hypohydrosis, hyperhydrosis, acanthosis, hyperkeratosis, skin peeling.

- Historical data on serum creatinine (µmol/L or mg/dL) (if available) within the 2 years prior to screening (e.g., 2 values per year 6 months apart from each other).

7.2.1.2 Data to be collected for screening failure subjects

- A minimal set of screening failure information is required to ensure transparent reporting of screening failure subjects.

For subjects who failed screening, the following data will be recorded in the eCRF:

- Age, sex, race, and ethnicity (if allowed in the country);
- Inclusion criteria not met and/or exclusion criteria met;
- FD history [see Section 7.2.1.1];
- FD-specific therapies [see Section 5.2.5.1];
- SAEs.

7.2.2 Efficacy assessments

7.2.2.1 Use of an electronic diary

All PROs collected on a daily basis and during a site visit will be recorded in an eDiary. The data from the eDiary will be automatically transferred to the eDiary vendor's central server. Once the data have been transferred to the vendor's central server, the site staff, the eDiary vendor, and sponsor representatives can access them in a view-only mode. More details on eDiary data transfer procedures and data access are provided in Section 11.1.

Each subject will be provided with a portable eDiary with a touch screen interface. The subjects will be trained by study personnel at the time the subject is given access to the eDiary device (i.e., during the screening visit). A training version of the eDiary will be available to support the training session. The training version of the eDiary will allow a subject to complete every questionnaire he/she will encounter during the study, and to practice until he/she feels comfortable with the eDiary. Subjects must be re-trained during subsequent site visits or telephone calls if needed.

The subject will complete the eDiary according to the protocol schedule. The subject should preferably complete the eDiary at approximately the same time every evening for those questionnaires collected on a daily basis ("evening diary"). Reminders will be programmed for scheduled diary entries (like the evening diary) and will occur when the diary is due for completion but data has not yet been entered. The subject will be instructed to bring his/her eDiary at the site in order to complete the site visit PROs (i.e., SF-36v2™, BPI-SF, PGIS-P, PGIC-PS, PGIS-D, PGIC-DS, CESD-R-20).

When the subject has completed all the screens belonging to a diary (e.g., evening diary) or a site visit PRO (e.g., BPI-SF), he/she will have to confirm his/her answers. Once the subject will submit the confirmation screen, he/she will no longer be able to access or change the data entered in that diary or PRO.

The subject will return the eDiary to the site personnel at the EOT visit or at the last PTOP visit (whichever is last).

7.2.2.2 Efficacy assessments collected on a daily basis in the electronic diary

7.2.2.2.1 Assessment of neuropathic pain

Subjects will be asked to rate on a daily basis their neuropathic pain in the eDiary starting at the Screening visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP).

Subjects will rate their neuropathic pain intensity on an NRS-11 (0 – no neuropathic pain to 10 – neuropathic pain as bad as you can imagine) to respond to the modified BPI-SF3 in the eDiary:

The following question is about the neuropathic pain you may have because of your Fabry disease.

This type of pain usually feels like ***burning, shocks or shooting, stabbing, tingling, and/or pins and needles*** *in your hands and feet. You may have constant or intermittent pain with variable intensity. You may also get attacks of intense, excruciating pain that starts in the hands and feet and spreads to other parts of the body.*

Your pain may occur randomly. It may also be triggered by heat or cold, weather change, being sick or having a fever, diet, stress, tiredness and/or physical activity.

"Please rate your neuropathic pain (e.g., pain that feels like burning, shocks or shooting, stabbing, tingling, and/or pins and needles) in your hands and feet by selecting the number that best describes your neuropathic pain at its WORST in the last 24 hours."

Risk minimization of potential bias

Subjects must not receive any of the following information:

- Pain intensity randomization criterion (inclusion criterion #9 [Section 4.3]);
- Computation rules and time windows used to derive the baseline modified BPI-SF3 scores [see Section 10.2.1];
- Individual baseline modified BPI-SF3 score.

The investigator and medical staff should not communicate any of the above information to the subject or any other parties who could be potentially in contact with the subject (e.g., subject's general practitioner).

7.2.2.2.2 Assessment of abdominal pain intensity

The subjects will be asked to rate on a daily basis their abdominal pain in the eDiary starting at the screening visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP).

Subjects will rate their abdominal pain intensity on an NRS-11 (0 – no pain to 10 – worst imaginable pain) to respond to the following question in the eDiary:

- "Please rate your abdominal pain by selecting the number that best describes your abdominal pain at its WORST in the last 24 hours."

7.2.2.2.3 Assessment of number and consistency of bowel movements

The subjects will be asked to record on a daily basis the number and consistency of bowel movements starting at the Screening visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP).

The subject will answer the following question in the eDiary:

- "How many bowel movements did you have in the last 24 hours?"

The subject will then have to indicate the BSS type of each bowel movement [Appendix 2].

The BSS [Appendix 2] is a medical aid designed to classify the form of human feces into seven categories. It provides a visual and verbal description of stool consistency and form.

The use of the BSS is not covered by a license.

7.2.2.2.4 Risk minimization for missing data

A series of measures will be implemented to minimize the risk of missing data entered on a daily basis by the subject:

- Data will be collected in an eDiary: when compared with collecting PRO on paper diary, electronic data collection has been shown to dramatically increase subject compliance [Lauritsen 2004];
- The subject will be trained on the use of the eDiary during the screening visit;
- The first 2 weeks of the screening period will allow the subject to fully familiarize themselves with the eDiary. Data collected during that time period will not be used for the setting of the baseline scores;
- The subjects will get audible and visual alarms and reminders to complete the diary on a daily basis;
- Data are transferred from the eDiary to the eDiary vendor's central server on a nightly basis [see Section 11.1] allowing the site staff, the sponsor and the eDiary CRO to remotely monitor the compliance of the subject;
- The site staff, and the sponsor will get e-mail alerts in the event of subject non-compliance. The study staff with the support of the eDiary vendor and the sponsor will ensure the subject is advised and re-trained (if needed) to limit the number of days with incomplete data collection. The main reason for not completing the diary will be collected and documented in the hospital charts;
- In the event of technical failure of the eDiary (excluding lack of eDiary recharging by the subject), a back-up plan includes provisioning all sites with back up eDiary and overnight shipment of the replacement eDiary to the subject.

In the event of premature study treatment discontinuation, the subject is requested (to the extent of possibility) to continue completing the eDiary until the originally scheduled Month 6 visit.

7.2.2.3 Brief Pain Inventory-Short Form

The full BPI-SF questionnaire will be completed by the subject at the randomization visit and at each subsequent site visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP). The questionnaire will be administered in an electronic format using the eDiary [see Section 7.2.2.1].

The BPI-SF is a validated, self-administered questionnaire, developed to assess the severity and impact of pain. The BPI-SF provides a direct and comprehensive means of measuring pain intensity, location of pain, and the extent to which pain interferes with the subject's

daily activities. The BPI was originally designed to assess pain in cancer subjects but has proven to work in other painful conditions including neuropathic pain and FD [Cleeland 2002].

The BPI-SF allows subjects to rate the severity of their pain (4 questions assessed on an NRS-11) and the degree to which their pain interferes with common dimensions of feeling and function (7 questions assessed on an NRS-11). Location of pain is also indicated on a body scheme by the subject.

A sample of the BPI-SF (in English) is provided in Appendix 3.

The sponsor has been granted a license agreement for the use of the BPI-SF.

7.2.2.4 Patient Global Impression of Severity

Two PGIS questionnaires will be completed by the subject at the randomization visit and at each subsequent site visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP): a PGIS-D and a PGIS-P. The questionnaires will be administered in an electronic format using the eDiary.

The PGIS questionnaires are self-administered 1-item questionnaire designed to assess subject's impression of disease severity (PGIS-D) and neuropathic pain severity (PGIS-P). Subjects will rate the overall severity of the disease and neuropathic pain symptoms over the past 7 days on a 4-point scale (1–4) scored as: "none", "mild", "moderate", or "severe".

Samples of the PGIS-D and PGIS-P questionnaires are provided in Appendix 4.

7.2.2.5 Patient Global Impression of Change

Two PGIC questionnaires will be completed by the subject starting at Month 1, and at each subsequent site visit until the Month 6 visit (Month 6 PTOP visit if subject is in the PTOP): a PGIC-DS and a PGIC-PS. The questionnaires will be administered in an electronic format using the eDiary.

The PGIC questionnaires are self-administered 1-item questionnaires designed to assess subject's impression of change since study treatment start in disease severity (PGIC-DS) and neuropathic pain severity (PGIC-PS). Subjects will rate their change since they started study treatment in the overall severity of the disease symptoms (PGIC-DS) and change in the overall severity of neuropathic pain (PGIC-PS) on 7-point scales (1 to 7) scored as: "very much improved," "much improved," "minimally improved," "no change," "minimally worse," "much worse," or "very much worse".

Samples of the PGIC-DS and PGIC-PS questionnaires are provided in Appendix 4.

7.2.2.6 Center for Epidemiologic Studies Depression Scale Revised

The CESD-R-20 will be completed by the subject at the randomization visit and Month 6 (Month 6 PTOP visit if subject is in the PTOP). The questionnaire will be administered in an electronic format using the eDiary.

The CESD was created in 1977 [Radloff 1977] and revised in 2004 (CESD-R-20 [Eaton 2004]). This self-report scale is well known and remains one of the most widely used instruments in the field of psychiatric epidemiology.

The 20 items in the CESD-R-20 scale measure symptoms of depression in nine different groups as defined by the American Psychiatric Association Diagnostic and Statistical Manual, fifth edition.

The total CESD-R-20 score is calculated as a sum of responses to all 20 questions. A score equal to or above 16 indicates a person at risk of clinical depression.

A sample of the CESD-R-20 (in English) is provided in Appendix 5.

The use of the CESD-R-20 is not covered by a license.

7.2.2.7 Echocardiography

A central echocardiography vendor (see echocardiography manual for contact details) will be used for all protocol-mandated echocardiographies, including re-tests, and echocardiographies performed at unscheduled visits.

A re-test may be performed if the result for an echocardiography is missing (e.g., unreadable echocardiography) or to repeat an abnormal echocardiography. A re-test does not need to be reported as an unscheduled visit [see Section 7.1.2].

Standard 2D/Doppler echocardiographies will be collected at the randomization visit, and Month 6 (Month 6 PTOP visit if subject is in the PTOP). Echocardiographies will be acquired by trained sonographers/technicians using the echocardiography Image Acquisition Guidelines. The sonographers/technicians will have to be qualified by the central echocardiography vendor prior to any acquisition of echocardiographies in a study subject. Whenever possible, the same sonographer/technician should acquire all echocardiographies performed in one subject. The site personnel will electronically transmit the echocardiographies to the central echocardiography vendor for central reading.

The overview of the assessment criteria and read procedures used by the echocardiography vendor will be described in a charter.

Central echocardiography reports will be provided by the central echocardiography vendor to the investigator/delegate. In the event of specific echocardiography abnormalities, the central echocardiography vendor will alert the sponsor and the site personnel. All

echocardiography reports must be reviewed, signed, and dated by the investigator/delegate within 10 working days of receipt and filed with the source documentation.

Incidental clinically relevant findings on echocardiographies will be reported in the Medical History or as an AE in the eCRF, as applicable.

The measures/assessments evaluated by the central echocardiography vendor include but are not limited to:

- LVMI for height$^{2.7}$ (g/m$^{2.7}$);
- Posterior wall thickness (mm);
- Left ventricular mean wall thickness (mm);
- LVEF (%);
- Left ventricular end diastolic volume (mL);
- Left ventricular end systolic volume (mL);
- Left atrial volume (mL).

Details on echocardiography procedures (recording, transfer of data and reporting) will be provided in the echocardiography manual.

7.2.2.8 Exit interview (US and Canada)

English-speaking subjects in US and Canada will be contacted by an external CRO to set an interview as soon as possible after the end of study treatment phase or EOT visit, ideally within 2 weeks. The interviews will be conducted via telephone by qualified staff from the CRO following the semi-structured interview guide ("Discussion Guide for the Exit Interview"). The interviews will take approximately 45 minutes and will be audio-recorded and then transcribed; the audio files and transcripts will be de-identified and securely transferred to the sponsor by the CRO.

The purpose of exit interviews is to further explore what constitutes meaningful changes on the neuropathic pain (assess with modified BPI-SF3) and GI symptom (diarrhea and abdominal pain) questions from a subject's perspective. Subjects will also be asked about meaningful change on the PGIC and PGIS questions.

Further details are provided in the separate documents "Exit interview sub-study protocol" and "Discussion Guide for the Exit Interview". After the first 5 interviews are completed, the CRO staff will meet with the sponsor to review the de-identified data, discuss the findings at that stage, and adjust the interview process, if needed.

The interviews will be analyzed and interpreted by the CRO and reported in a separate report.

7.2.3 Safety assessments

The definitions, reporting and follow-up of AEs/SAEs and pregnancies are described in Section 9.

Unless otherwise specified, the date of each safety assessment will be collected in the eCRF.

7.2.3.1 Physical examination

Physical examination at screening includes the examination of the general appearance (heart, lungs, abdomen, skin, extremities, eyes, ears, nose, throat, lymph nodes, nervous system, etc.). At subsequent visits, physical examination includes, as a minimum, the examination of skin, heart, lungs, eyes, and ears.

Each physical examination must include a complete skin examination performed by the investigator/delegate. In the event of findings other than regular skin findings relevant to FD (e.g., angiokeratoma, hyperkeratosis), a dermatologist will conduct further examination per local standard practice, including performing skin biopsies if required to rule out or confirm a diagnosis.

Other examinations will be performed if indicated, based on medical history and/or symptoms.

Information for all physical examinations must be included in the source documentation at the study site.

The physical examination will be reported in the eCRF as either normal or abnormal; in the latter case, the investigator should specify whether it is clinically relevant. Clinically relevant physical abnormalities present at the time of signing of the ICF must be reported on the medical history or Fabry disease forms of the eCRF. Physical examination findings, which meet the definition of an AE [Section 9.1.1] and are made after signing of the ICF, must be recorded on the AE form of the eCRF.

7.2.3.2 Vital signs

HR, SBP, and DBP will be measured at each visit in a supine or sitting position and recorded in the eCRF. It is recommended to allow the subject to rest for at least 5 minutes, and to use the same arm and position (supine or sitting) throughout the study for an individual subject.

The date and exact actual clock time of vital sign assessments will be entered in the eCRF.

7.2.3.3 Weight and height

Height will be measured without shoes at screening.

Body weight will be measured in indoor clothing but without shoes at screening and each visit thereafter.

Body weight and height data will be recorded in the eCRF.

7.2.3.4 Electrocardiogram assessment

A standard pre-dose 12-lead ECG will be performed at all scheduled visits. At the Month 1 visit, 2 additional 12-lead ECGs will be performed 2 h and 4 h after study treatment administration.

A re-test may be performed if the result for an ECG is missing or to repeat an abnormal ECG. A re-test does not need to be reported as an unscheduled visit [see Section 7.1.2].

ECGs will be performed with the subject in a fully rested supine position after the subject has been allowed to rest for a minimum of 5 minutes prior to the measurement. The date and exact clock time of ECGs will be entered in the eCRF.

A central ECG vendor (see ECG manual for contact details) will be used for the evaluation of all protocol-mandated ECGs, including re-tests due to ECG abnormalities and ECGs performed at unscheduled visits. The site personnel will electronically transmit the ECGs to the central ECG vendor for central reading.

The following variables will be evaluated by the central ECG vendor: HR (bpm), PR (ms), QRS (ms), QT (ms), QTc according to Bazett's formula (QTcB) (ms), QTcF (ms) and any ECG findings. QTc (ms) will be calculated according to Bazett's and Fridericia's formula (QTcB = QT/[RR]½ and QTcF = QT/[RR]⅓, respectively).

ECG reports will be provided by the central ECG vendor to the investigator/delegate. If specific (pre-defined in the ECG manual) ECG abnormalities are observed, the central ECG vendor will alert the sponsor and the site personnel.

All ECG reports must be reviewed, signed and dated by the investigator or delegate within 10 working days of receipt and filed with the source documentation. The investigator/delegate must indicate on the ECG report whether abnormal values or findings are considered clinically relevant or not. Clinically relevant ECG findings that are known at the time of signing of the ICF must be recorded in the medical history or FD forms of the eCRF. Any clinically relevant ECG abnormalities detected after signing of the ICF must be reported as an AE or SAE as appropriate [see Section 9], and must be followed until the finding returns to normal, is considered stable, or until the change is no longer clinically relevant.

Details on ECG procedures (recording, transfer of data and reporting) will be provided in the ECG manual.

7.2.4 Laboratory assessments

7.2.4.1 Type of laboratory

A central laboratory (see laboratory manual for contact details) will be used for all protocol-mandated laboratory tests (with the exception of urine pregnancy tests), including re-tests due to laboratory abnormalities and laboratory tests performed at unscheduled visits.

Local laboratory results of the parameters described in Section 7.2.4.2 will only be collected in exceptional circumstances (e.g., hospitalization of the subject due to a medical emergency, and missing central laboratory results from a scheduled or unscheduled visit). The local laboratory results (with the corresponding normal ranges) must be recorded in the eCRF.

If two or more consecutive central laboratory samples are lost or cannot be analyzed for whatever reason, the investigator will collect an additional sample as soon as possible to repeat the analysis, unless a local laboratory sample was collected within the same time-window and these test results are available.

Laboratory reports will be provided by the central laboratory to the investigator/delegate. In the event of specific (pre-defined in the laboratory manual) laboratory abnormalities, the central laboratory will alert the sponsor and the site personnel.

All laboratory reports must be reviewed, signed and dated by the investigator/delegate within 10 working days of receipt and filed with the source documentation.

A re-test may be performed if the laboratory result is missing or to repeat an abnormal laboratory result. A re-test does not need to be reported as an unscheduled visit [see Section 7.1.2].

The investigator/delegate must indicate on the laboratory report whether abnormal values are considered clinically relevant or not. Clinically relevant laboratory findings that are known at the time of signing of the ICF must be recorded in the medical history or FD forms of the eCRF. Any clinically relevant laboratory abnormalities as per investigator judgment detected after signing of the ICF must be reported as an AE or SAE as appropriate [see Section 9], and must be followed until the value returns to within the normal range or is considered stable and no longer clinically relevant.

Details about the collection, sampling, storage, shipment procedures, and reporting of results and abnormal findings can be found in the laboratory manual.

7.2.4.2 Laboratory tests

Unless otherwise specified, the date of each laboratory sample will be collected in the eCRF.

7.2.4.2.1 Blood tests

Blood samples will be drawn under fasted[b] condition (i.e., skipping breakfast) and when applicable before the morning administration of the study treatment at all scheduled and unscheduled visits.

Hematology:

- Hemoglobin (g/L);
- Hematocrit (%);
- Erythrocyte count (reticulocyte count) (10^{12}/L);
- Leukocyte count with differential counts (10^9/L);
- Platelet count (10^9/L).

Blood chemistry:

- ALT (U/L), AST (U/L), alkaline phosphatase (U/L);
- Total and direct bilirubin (µmol/L);
- Creatinine (µmol/L) measured using an enzymatic assay;
- eGFR* (mL/min/1.73 m^2);
- Blood urea nitrogen (mmol/L);
- Uric acid (µmol/L);
- Albumin (g/L), protein total (g/L);
- Glucose (mmol/L), HbA1c (%);
- Cholesterol (mmol/L), triglycerides (mmol/L);
- Sodium, potassium, chloride, calcium (mmol/L);
- N-terminal pro-brain natriuretic peptide (ng/L).

*eGFR will be estimated using the CKD-$EPI_{creatinine}$ equation [Levey 2009] as follows:

$eGFR_{creatinine} = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ (if female) × 1.159 (if black)

where:

Scr = serum creatinine in mg/dL
$\kappa = 0.7$ for females, $\kappa = 0.9$ for males,

[b] Subjects must not be requested to be in a fasted state (for the purpose of the study) at the screening visit unless the ICF has already been fully signed.

$\alpha = -0.329$ for females, $\alpha = -0.411$ for males,
min = the minimum of Scr /κ or 1, max = the maximum of Scr /κ or 1

Cardiac enzymes:

- High sensitivity troponin T (µg/L).

α-Galactosidase A:

An extra blood sample will be collected at the screening visit and randomization visit to assess the α-GalA in leukocytes (nmol/h/mg of protein). The samples will be shipped to the central laboratory for analysis.

7.2.4.2.2 Urinalysis

A midstream, clean-catch urine specimen will be collected for dipstick analysis and determination of creatinine (µmol/L), albumin (mg/L), UACR (mg/g) at all scheduled and unscheduled visits. The collected urine will be transferred into standard urine collection tube for shipment to central laboratory.

7.2.4.2.3 Pregnancy tests

A serum pregnancy test for women of childbearing potential will be performed at screening. A urine pregnancy test will be performed at randomization and monthly thereafter with locally approved kits provided by the site. If pregnancy is suspected during the study, a serum pregnancy test must be performed immediately.

The results of the urine pregnancy tests will not be collected in the eCRF. Serum pregnancy tests will be sent to the central lab for analysis and the result will be sent to the investigator/delegate (see laboratory manual for details on collection, sampling, storage, shipment procedures, and reporting of results).

Reporting procedures of pregnancy are described in Section 9.4.1.

7.2.4.2.4 Fecal analyses

A fecal sample will be collected at the screening visit in subjects who report diarrhea symptoms to assess subject eligibility (exclusion criteria #8b, see Section 4.4).

If no stool is available on the day of the screening visit, the subject will be provided with collection material and instructed by the site personnel how to collect, store and ship a stool sample. The subject will record the date and time at which the sample was produced.

The collected fecal samples will be transferred by the study personnel as described in the laboratory manual and study specific laboratory flowchart.

Stool exam will include calprotectin test, fecal occult blood test, stool culture, and test for ova and parasites.

7.2.5 Quality of life assessments

7.2.5.1 36-Item Short Form Health Survey Version 2

The SF-36v2™ questionnaire (SF-36v2™ Health Survey© 1996, 2000 by Medical Outcomes Trust and Quality Metric Incorporated) is used to assess the subject's QoL. The SF-36v2™ will be completed by the subject at the randomization visit and Month 6 (Month 6 PTOP visit if subject is in the PTOP). using the eDiary [see Section 7.2.2.1].

In the SF-36v2™ questionnaire, subjects are instructed to rate their health and capacity to perform activities of daily living in 8 domains including physical functioning, physical role limitations and mental health during the last 4 weeks. Raw domain scores are determined and transformed to a 0–100 scale as described in the SF-36v2™ manual [Maruish 2011]. Individual domain scores are used to determine the physical and mental component summary scores as described in the SF-36v2™ manual [Maruish 2011].

A sample of the SF-36v2™ (in English) is provided in Appendix 6.

The sponsor has been granted a license agreement for the use of the SF-36v2™ questionnaire. The individual questionnaires will be completed only in countries for which validated translations are available.

7.2.6 Pharmacokinetic assessments

7.2.6.1 Pharmacokinetic assessments

Pre-dose blood samples for determination of PK will be collected for all subjects at the Month 1 visit and all subsequent visits to provide information about the study treatment exposure in the target population.

In addition, post-dose (0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 6 h, 8 h, 10 h, and 12 h) blood samples will be collected at the Month 1 visit in a subset of 36 subjects to explore the 12 h PK profile of lucerastat and if necessary, to identify its potential metabolites.

7.2.6.2 Sampling, labeling, storage and shipment

The date and exact actual clock time of collection of each blood sample will be entered in the eCRF as well as the exact dates and time of the study treatment administration prior to and after blood draw.

Details about the collection, sampling, storage, and shipment procedures can be found in the laboratory manual.

7.2.6.3 Bioanalysis

The analysis of lucerastat and if needed, possible metabolites in plasma will be performed using a validated liquid chromatography coupled to tandem mass spectrometry assay. The foreseen limit of quantification of lucerastat is 50 ng/mL.

The analysis of lucerastat and, if needed, its possible metabolites in plasma will be performed by the Idorsia bioanalytical laboratory. To minimize the possibility of systematic unblinding, the values of the PK parameters will not be communicated to the investigator and study personnel, subjects, CRAs, and sponsor and vendor/CRO personnel involved in the conduct of the study until the study database is locked.

The samples will be destroyed upon signature of the CSR and if applicable, the metabolite profiling report.

7.2.7 Biomarker assessments

7.2.7.1 Biomarkers of Fabry disease and their metabolic precursors

Plasma and urinary samples for analysis of biomarkers of FD and their metabolic precursors will be collected at all scheduled visits (pre-dose). The date and exact actual clock time of collection of each blood sample and urine sample will be entered in the eCRF.

Gb3 and lysoGb3, along with the Gb3 metabolic precursors GlcCer and LacCer will be quantified in plasma. Gb3 and lysoGb3 will be quantified in urine and normalized to creatinine.

A central laboratory (see laboratory manual for contact details) will be used for all protocol-mandated biomarker assessments. To minimize the possibility for systematic unblinding, biomarker laboratory results will not be communicated to investigator and study personnel, the subjects, CRAs, and any sponsor personnel and vendor/CRO personnel involved in the conduct of the study until the study database is locked.

7.2.7.2 Other non-genetic research samples

Extra plasma, serum and urine samples will be collected at trough at the randomization visit, Month 6 visit or EOT visit for additional non-genetic analyses including analysis of biomarkers that characterize FD or FD treatment characteristics (e.g., measurement of levels of antibodies against ERT, other analysis may be added after study closure, if scientific rationale has become available through new research). Those samples may be stored in the sponsor's research biorepository for a maximum of 15 years. Study participants may request these samples be destroyed at any time.

No genetic analysis will be conducted.

Details about the collection, sampling, storage and shipment procedures can be found in the laboratory manual.

8 STUDY COMPLETION AND POST-STUDY TREATMENT / MEDICAL CARE

8.1 Study completion as per protocol

Subjects who complete the 6-month, double-blind study treatment period, and if applicable FU1 and FU2, are considered to have completed the study as per protocol.

8.2 Premature withdrawal from study

Subjects may voluntarily withdraw from the study without justification for any reason at any time. Subjects are considered withdrawn if they state an intention to withdraw further participation in all components of the study (i.e., withdrawal of consent), die, or are lost to follow-up. If a subject withdraws consent, no further data will be collected in the eCRF from the date of withdrawal onward. The investigator may withdraw a subject from the study (without regard to the subject's consent) if, on balance, he/she believes that continued participation in the study would be contrary to the best interests of the subject. Withdrawal from the study may also result from a decision by the sponsor for any reason, including premature termination or suspension of the study.

Subjects are considered as lost to follow-up if all repeated attempts by the investigator to communicate with the individual have failed. The site must take preventive measures to avoid a subject being lost to follow-up (e.g., document different ways of contact such as telephone number, home address, email address, person to be contacted if the subject cannot be reached). If the subject cannot be reached, the site must make reasonable repeated efforts to contact the subject, document all attempts (date, time and type of contact made), and enter the loss of follow-up information into the eCRF. The following methods must be used: at least 3 contacts (e.g., telephone calls, or e-mails) must be placed to the last available telephone number or e-mail address) and 1 registered letter must be sent by post to the last available home address. Additional methods may be acceptable if they are compliant with local rules/regulations (e.g., a visit by site personnel to the subject's home), respecting the subject's right to privacy. If the subject is still unreachable after all contact attempts listed above he/she will be considered to be lost to follow-up.

The reason for premature withdrawal from the study must be recorded in the eCRF.

If for whatever reason (except death or loss-to-follow-up) a subject is withdrawn from the study, the investigator should make best efforts to schedule a last appointment / telephone call to assess the safety and well-being of the subject, collect unused study treatment and discuss follow-up medical care. Data obtained during this last appointment / telephone call will be recorded in the subjects' medical records but it will not be collected in the eCRF.

The investigator must provide follow-up medical care for all subjects who are prematurely withdrawn from the study, or must refer them for appropriate ongoing care, as described in Section 8.4.

8.3 Premature termination or suspension of the study

The sponsor reserves the right to terminate the study at any time globally or locally. Investigators can terminate the participation of their site in the study at any time.

If a study is prematurely suspended or terminated, the sponsor will promptly inform the investigators, the IECs/IRBs, and health authorities, as appropriate, and provide the reasons for the suspension or termination.

If the study is suspended or prematurely terminated for any reason, the investigator — in agreement with the sponsor — must promptly inform all enrolled subjects and ensure their appropriate treatment and follow-up, as described in Section 8.4. The sponsor may inform the investigator of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the subjects' interests.

In addition, if the investigator suspends or terminates a study without prior agreement from the sponsor, the investigator must promptly inform the sponsor personnel and the IEC/IRB, and provide both with a detailed written explanation of the termination or suspension.

If the IEC/IRB suspends or terminates its approval / favorable opinion of a study, the investigator must promptly notify the sponsor personnel and provide a detailed written explanation of the termination or suspension.

8.4 Medical care of subjects after study completion / withdrawal from study

After the subject's study completion or premature withdrawal from the study, whichever applies, the investigator/delegate will explain to subjects what treatment(s) / medical care is necessary and available according to local medical practice and applicable guidelines. Such care may include:

- Use of drugs which were forbidden during concomitant study treatment administration;
- Enrollment in the OLE of this study conducted under a separate protocol, only for those subjects who have completed the 6-month double-blind treatment period, providing they fulfill the specific eligibility criteria of this separate study.

Female subjects of childbearing potential and fertile male subjects will be reminded of the contraception requirements as described in Section 4.5 and 4.6, respectively.

9 SAFETY DEFINITIONS AND REPORTING REQUIREMENTS

9.1 Safety definitions

9.1.1 Definition of adverse events

An AE is any untoward medical occurrence, i.e., any unfavorable and unintended sign, including an abnormal laboratory finding, symptom, or disease that occurs in a subject

during the course of the study, whether or not considered by the investigator as related to study treatment.

AEs include:

- Exacerbation of a pre-existing disease if considered medically relevant. The evolution of the FD disease condition including worsening of symptoms (e.g., neuropathic pain, GI symptoms) will be evaluated and reported as AE only if considered medically significant based on investigator judgment;
- Increase in frequency or intensity of a pre-existing episodic disease or medical condition;
- Disease or medical condition detected or diagnosed during the course of the study even though it may have been present prior to the start of the study;
- Continuous persistent disease or symptoms present at study start that worsen following the signing of the ICF;
- Abnormal change on physical examination, ECG findings, if they represent a clinically significant finding that was not present at study start or worsened during the course of the study as per investigator medical assessment;
- Laboratory test abnormalities if they represent a clinically significant finding (symptomatic or not) which was not present at study start or worsened during the course of the study as per investigator judgment, led to dose reduction, interruption or permanent discontinuation of study treatment.

9.1.2 Definition of serious adverse events

An SAE is defined by the ICH guidelines as any AE fulfilling at least 1 of the following criteria:

- Fatal;
- Life-threatening: 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 that hypothetically might have caused death had it been more severe;
- Requiring in-patient hospitalization or prolongation of existing hospitalization;
- Resulting in persistent or significant disability or incapacity;
- Congenital anomaly or birth defect;
- Medically significant: refers to important medical events that may not immediately result in death, be life-threatening, or require hospitalization but may be considered medically significant based upon appropriate medical judgment, as they may jeopardize the subject, and/or may require medical or surgical intervention to prevent one of the outcomes listed in the definitions above.

The following reasons for hospitalization are not considered as SAEs:

- Hospitalization for cosmetic elective surgery, or social and/or convenience reasons;
- Hospitalization for pre-planned (i.e., planned prior to signing ICF) surgery or standard monitoring of a pre-existing disease or medical condition that did not worsen, e.g., hospitalization for coronary angiography in a subject with stable angina pectoris.

However, complications that occur during hospitalization are AEs or SAEs (e.g., if a complication prolongs hospitalization).

9.1.3 Definition of suspected unexpected serious adverse reactions

The expectedness of an SAE is determined by the sponsor according to the reference safety information (RSI) section provided in the most recent version of the IB.

Any SAE that is assessed as related and unexpected against the RSI is known as a SUSAR.

9.1.4 Intensity of adverse events

The intensity of AEs is graded on a three-point scale — mild, moderate, severe — as follows:

- **Mild**

The event may be noticeable to the subject. It does not usually influence daily activities, and normally does not require intervention.

- **Moderate**

The event may make the subject uncomfortable. Performance of daily activities may be influenced, and intervention may be needed.

- **Severe**

The event may cause noticeable discomfort and usually interferes with daily activities. The subject may not be able to continue in the study, and treatment or intervention is usually needed.

A mild, moderate, or severe AE may or may not be serious [see Section 9.1.2]. These terms are used to describe the intensity of a specific event. Medical judgment should be used on a case-by-case basis.

Seriousness, rather than intensity assessment, determines the regulatory reporting obligations [see Section 9.3.2].

9.1.5 Relationship to study treatment

Each AE/SAE must be assessed by the investigator as to whether or not there is a reasonable possibility of causal relationship to the study treatment, and reported as either related or unrelated.

9.1.6 Relationship to protocol-mandated procedure

An AE/SAE is defined as related to protocol-mandated procedure if it appears to have a reasonable possibility of a causal relationship to either the study design or to a protocol-mandated procedure.

The determination of the likelihood that a protocol mandated procedure caused the AE/SAE will be provided by the investigator.

9.2 Time period and frequency for adverse event / serious adverse event assessment and follow-up

The occurrence of an AE/SAE may come to the attention of study personnel during study visits, telephone calls or interviews of study participants presenting for medical care.

At each study visit (scheduled or unscheduled), the investigator will inquire about the occurrence of AE/SAEs since the last visit.

9.2.1 Follow-up of adverse events

AEs still ongoing at the EOS visit must be followed up until resolution, are no longer considered clinically relevant or until stabilization.

9.2.2 Follow-up of serious adverse events

SAEs still ongoing at the EOS visit must be followed up until resolution, stabilization, or until the event outcome is provided.

9.3 Reporting procedures

9.3.1 Reporting of adverse events

All AEs with an onset date after signing of the ICF and up to 30 days after study treatment discontinuation or up to the last visit of the PTOP period (whichever is the latest) must be recorded on specific AE forms of the eCRF.

Information to be collected in an AE form in the eCRF includes date of onset, action taken with the study treatment, outcome of AE, date of resolution (if applicable) and PI's assessment of intensity, and relationship to study treatment, study design or protocol mandated procedures.

Information on worsening of intensity will be collected on a new AE form. If the AE lessens in intensity, no change in the severity is required to be reported.

Follow-up information on ongoing AE obtained after the subject's EOS visit will not be collected in the eCRF.

9.3.2 Additional reporting procedure for serious adverse events

All SAEs must be reported by the investigator to the sponsor's Global Drug Safety department within 24 hours of the investigator's first knowledge of the event.

All SAEs occurring after signing of the ICF up to 30 days after study treatment discontinuation or up to the last visit of the PTOP period (whichever is the latest) must be recorded on an SAE form, regardless of the investigator-attributed causal relationship with study treatment or study-mandated procedures.

The SAE forms must be sent to the sponsor's Global Drug Safety department (see contact details on the SAE form). The investigator must complete the SAE form in English, and must assess the event's causal relationship to the study treatment.

Any relevant information from source documents regarding the SAE, e.g., hospital notes or discharge summaries, etc., must be summarized on the SAE form.

Follow-up information about a previously reported SAE must also be reported within 24 hours of receiving it. The sponsor's Global Drug Safety personnel may contact the investigator to obtain further information.

If the subject is hospitalized in a hospital other than that of the study site, it is the investigator's responsibility to contact this hospital to obtain all SAE-relevant information and documentation.

New SAEs occurring after the 30-day follow-up period or after the last visit of the PTOP period (whichever is the latest) must be reported to the sponsor's Global Drug Safety department within 24 hours of the investigator's knowledge of the event, **only** if considered by the investigator to be causally related to previous exposure to the study treatment.

9.4 Pregnancy

If a woman becomes pregnant while on study treatment, study treatment must be discontinued [see Section 5.1.10.1].

The investigator must counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus.

9.4.1 Reporting of pregnancy

Any pregnancy occurring in a female subject after signing of the ICF and up to 30 days following study treatment discontinuation must be reported to the sponsor's Global Drug Safety department within 24 hours of the investigator's knowledge of the event.

Any pregnancy occurring in the female partner of a male subject during the treatment period and up to the EOS visit or FU2 visit (whichever is first) must be reported to the sponsor's Global Drug Safety department within 24 hours of the investigator's knowledge of the event.

Pregnancies must be reported on the sponsor Pregnancy form, which is faxed to the sponsor's Global Drug Safety department (see contact details provided on the Pregnancy form).

The investigator must complete the Pregnancy form in English.

9.4.2 Follow-up of pregnancy

Any pregnancies must be followed-up to their conclusion and the outcome must be reported to the sponsor's Global Drug Safety department.

Any AE associated pregnancy of a female subject occurring up to the EOS visit must be reported on separate AE forms in the eCRF.

Any SAE occurring during the pregnancy must be reported on an SAE form as described in Section 9.3.2.

9.5 Reporting of study treatment overdose, misuse, abuse and medication errors

Study treatment overdose (defined as higher than the dose of study treatment prescribed), and study treatment errors will be reported as an AE when associated with signs or symptoms.

In addition, study treatment errors must be documented in the study drug log of the eCRF.

Misuse and abuse of the study treatment will be reported as an AE/SAE as determined.

9.6 Study safety monitoring

Study safety information (AEs, SAEs, laboratory values, ECGs, vital signs, and study-specific examinations as required) is monitored and reviewed on a continuous basis by the sponsor (in charge of ensuring subjects' safety as well as data quality).

The sponsor may request additional data pertaining to the diagnostic work-up of an AE or SAE (e.g., medical imaging, local laboratory values) for the purpose of monitoring safety. Such additional data may be shared with external experts.

10 STATISTICAL METHODS

10.1 Analysis sets

10.1.1 Screened Analysis Set

The Screened Analysis Set includes all subjects who have given informed consent to participate in the study and have a subject number.

10.1.2 Full Analysis Set

The Full Analysis Set (FAS) includes all subjects randomized to either lucerastat or placebo. In order to adhere to the intent-to-treat principle as much as possible:

- Subjects will be evaluated according to their assigned study treatment (not actual treatment received) and stratum information as recorded in the IRT system;
- Unless otherwise stated, all available efficacy data for the primary and secondary endpoints will be included in the analyses up to the planned analysis time point, regardless of study treatment discontinuation and/or switches to alternative FD treatments.

10.1.3 Modified Full Analysis Set

A modified FAS (mFAS) will be defined by including subjects from the FAS who took at least one dose of study treatment. As in the FAS, subjects will be analyzed based on the assigned study treatment.

10.1.4 Modified Full Analysis Set-gastrointestinal symptoms

The mFAS-gastrointestinal symptoms (mFAS-GIS) comprises all subjects from the mFAS who, during the 4 weeks prior to randomization, have experienced:

- Abdominal pain of moderate to severe intensity at baseline defined as an average abdominal pain intensity score ≥ 3 on a NRS-11 scale; and/or
- Diarrhea at baseline defined as having at least 1 stool of a BSS consistency Type 6 or 7 on at least 8 days.

10.1.5 Per-Protocol Analysis Set

The Per-Protocol Analysis Set (PPS) includes all subjects from the mFAS without protocol deviations occurring prior to Month 6, which could affect the analysis of the primary endpoint variable.

The precise reasons for excluding subjects from the PPS will be fully defined and documented in the statistical analysis plan (SAP) before breaking the randomization blind.

10.1.6 Safety Set

The Safety Set (SAF) includes all subjects who received at least 1 dose of study treatment (as recorded in the eCRF). Subjects will be analyzed based on the treatment received. In the situation of accidental dispensation of both study treatments to the same subject, the subject will be counted as on lucerastat for the whole study.

10.1.7 Other analysis sets

Other analysis datasets will be defined in the SAP (or corresponding SAPs), e.g., PK set, sub-study sets, and subgroups of interest.

10.1.8 Usage of the analysis sets

The mFAS and the PPS are used for the analysis of the primary and secondary endpoint related to Gb3. Results based on the PPS will supplement those based on the mFAS to assess the robustness of the treatment effect. The primary and secondary endpoint analyses will also be produced on the FAS as sensitivity analyses if the FAS and mFAS differ by at least 5 subjects (approximately 5% of the total sample size).

The secondary endpoints related to abdominal pain and diarrhea will be assessed on the mFAS-GIS.

All safety data will be analyzed using the SAF.

The usage of other analysis sets will be described in the SAP.

10.2 Variables

In this section, variables are defined in detail for the primary and secondary efficacy endpoints and for the safety endpoints. All variables for all other endpoints will be defined in detail in the SAP.

10.2.1 Primary efficacy variable

Baseline and Month 6 modified BPI-SF3 scores will be obtained by averaging the weekly modified BPI-SF3 scores over the 4 weeks prior to the corresponding visit of interest (randomization visit and Month 6 visit, respectively). At least 4 of the 7 daily scores should be non-missing to derive a valid weekly modified BPI-SF3 score and at least 3 valid weekly scores should be non-missing to derive a valid score for the relevant time period.

All available daily scores will be used to derive the scores regardless of study treatment discontinuations or introductions of new medications.

In addition, intermediate monthly modified BPI-SF3 scores from Month 1 to Month 5 will be derived with the same validity conditions. The selection of daily scores over 4 weeks for these time points will be described in the SAP.

10.2.2 Secondary efficacy variables

The following 3 secondary efficacy endpoint variables are considered:

- Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline;
- Change from baseline to Month 6 in the number of days with at least 1 stool of a BSS consistency Type 6 or 7 in subjects with GI symptoms at baseline;
- Change from baseline to Month 6 in plasma Gb3.

10.2.2.1 Change from baseline to Month 6 in the 11-point numerical rating scale score of "abdominal pain at its worst in the last 24 hours" in subjects with gastrointestinal symptoms at baseline

Using the abdominal pain intensity scores collected daily, the variable "change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" will be derived using the same rules as those described for the primary efficacy variable [see Section 10.2.1]. The variable will be derived only for those subjects with GI symptoms at baseline as defined in Section 10.1.4.

10.2.2.2 Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale consistency Type 6 or 7 in subjects with gastrointestinal symptoms at baseline

Using the number of complete stool bowel movements with BSS consistency of Type 6 or 7 collected daily, the variable "change from baseline to Month 6 in the number of days with at least one stool of a BSS consistency Type 6 or 7" will be derived using the following rules:

The number of days with diarrhea at baseline and Month 6 will be calculated as the number of days observed with diarrhea over the 4 weeks prior to the corresponding visit of interest (randomization and Month 6, respectively) over the number of days with data available multiplied by 28, provided there are at least 21 measures available in the corresponding time period.

The variable will be derived only for those subjects with GI symptoms at baseline as defined in Section 10.1.4.

All available data will be used to derive the number of days with diarrhea regardless of study treatment discontinuations. In addition, the intermediate number of days with diarrhea from Month 1 to Month 5 will be derived with the same validity conditions. The selection of daily scores over 4 weeks for these time points will be described in the SAP.

10.2.2.3 Change from baseline to Month 6 in plasma globotriaosylceramide

Using the plasma Gb3 data collected at baseline and Month 6, the variable "change from baseline to Month 6 in Gb3" will be calculated as follows:

(Gb3 at Month 6 – Gb3 at baseline).

10.2.3 Safety variables

The variables described in this section are to be used for the derivation of the safety endpoints described in Section 6.2.

Treatment-emergent laboratory values, vital signs or AEs are those with an assessment or onset date/time ≥ start date/time of study treatment and ≤ 30 days after study treatment discontinuation.

Abnormalities for laboratory, vital signs and ECGs will be defined in the SAP.

Laboratory analyses are based on data received from the central laboratory as well as local laboratories.

All laboratory, vital signs and ECG data are taken into account regardless of whether they correspond to scheduled or unscheduled assessments.

10.3 Description of statistical analyses

All available data for each subject will be used in all statistical analyses unless otherwise specified.

10.3.1 Overall testing strategy

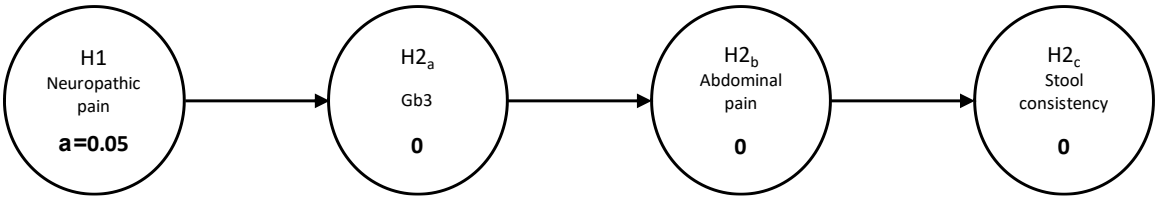
Comparisons of lucerastat vs placebo will be conducted for the primary and secondary endpoints assessed at Month 6.

The Type I error rate will be controlled at a two-sided alpha of 5% for the testing of the four null hypotheses associated with the primary and secondary endpoint comparisons employing a fixed-sequence statistical testing strategy in the following order:

1. Change from baseline to Month 6 in the "modified" BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours".
2. Change from baseline to Month 6 in plasma Gb3.
3. Change from baseline to Month 6 in the NRS-11 score of "abdominal pain at its worst in the last 24 hours" in subjects with GI symptoms at baseline;
4. Change from baseline to Month 6 in the number of days with at least one stool of a BSS consistency Type 6 or 7 in subjects with GI symptoms at baseline.

The order of the fixed-sequence statistical testing strategy is depicted in Figure 2. In the event that the null hypothesis is not rejected for an endpoint in the sequence, claims for statistical significance cannot be made for endpoints that follow in the sequence.

Figure 2 Fixed-sequence statistical testing strategy



The primary hypothesis related to H1 is initially assigned the local two-sided statistical significance level alpha = 0.05, whereas $H2_a$ through $H2_c$ from the secondary family of endpoints are assigned the local statistical significance level 0. If H1 is rejected, the local statistical significance level α is passed on to $H2_a$ and so on as long as hypotheses are rejected.

10.3.2 Analysis of the primary efficacy variable

10.3.2.1 Hypotheses

Hypotheses for the primary endpoint are formulated in terms of the mean differences in change from baseline to Month 6.

H_0: Lucerastat - Placebo = 0

is the null hypothesis that there is no difference between treatments.

H_A: Lucerastat - Placebo ≠ 0

is the alternative hypothesis that a difference in change from baseline to Month 6 exists between treatments.

The hypotheses for the secondary endpoints related to abdominal pain and plasma Gb3 are defined in the same way.

The null hypothesis for the secondary endpoint related to stool consistency is that both groups are from identical distributions while the alternative hypothesis is that the distributions differ with respect to location.

10.3.2.2 Primary statistical analysis

The primary statistical analysis will be performed on the mFAS, according to the intent-to-treat approach. All available data will be used regardless of occurrence of ICEs such as premature treatment discontinuation or changes in background medication.

The null hypothesis will be tested at the two-sided alpha level = 0.05, using the following method:

Missing data will be imputed applying a control-based multiple imputation (MI) assuming MNAR using the Copy Reference (CR) approach [Carpenter 2013]. Instead of imputing a single value for each missing observation, a set of values is generated from the model, resulting in as many distinct complete datasets without missing data. The imputation model includes the baseline value, the two stratification factors (sex and ERT treatment status at screening) and all post-baseline monthly scores up to Month 6. Missing data for subjects from both treatment arms will be imputed using data from the placebo arm. This approach assumes that subjects with missing data in the lucerastat arm have outcomes trending towards outcomes observed in the placebo arm, i.e., the imputations result in a treatment effect that gradually diminishes towards the placebo arm.

An analysis of covariance (ANCOVA) model will be used to analyze this endpoint on each imputed dataset. The following terms will be included in the model: baseline value, the two stratification factors (sex and ERT treatment status at screening), and the treatment group.

Uncertainty in the imputations will be reflected appropriately in the analysis by combining the results on each imputed dataset using Rubin's methodology [Rubin 1987].

The mean difference in changes from baseline to Month 6 between lucerastat and placebo together with its two-sided 95% CI and p-value will be reported.

10.3.2.3 Handling of missing data

Some missing data may occur in the daily measurements, possibly translating into missing monthly scores. In the next sections "missing data" will refer to missing monthly modified BPI-SF3 scores.

The missing data will be described via:

- Visual inspection of missing data patterns;
- Characterization of missing data patterns according to reason, (e.g., lack of efficacy, safety and tolerability, etc.).

10.3.2.4 Sensitivity analyses for missing data

The robustness of inferences from the primary endpoint analysis to deviations from its underlying modelling assumptions will be explored using the following sensitivity analyses:

- Model based analyses relying on the missing at random (MAR) assumptions:

Missing monthly modified BPI-SF3 scores will be imputed using a MI method [Rubin 1987] based on a model including the baseline modified BPI-SF3 score, stratification factors, all available post-baseline monthly scores up to Month 6 and treatment group. In contrast to the CR approach described in Section 10.3.2.2, treatment group is included in the imputation model and therefore, missing data for subjects from the lucerastat arm will be imputed using data from the lucerastat arm.

- Model based analyses relying on "missing not at random" (MNAR) scenarios:

  Missing monthly modified BPI-SF3 scores will be imputed using a MI assuming MNAR using the Jump to Reference (J2R) approach [Carpenter 2013]. The J2R approach assumes that subjects with missing data in the lucerastat group have outcomes similar to outcomes from the placebo group, starting from the point of monotone missing data. In contrast to CR, for subjects in the lucerastat group, non-missing earlier values are ignored in the prediction of later missing values. Therefore, the treatment difference from the lucerastat group in subjects with missing data disappears immediately after the point where missing data occurs.

10.3.2.5 Supportive analyses

A series of additional analyses based on the modified BPI-SF3 score data are presented below and will support the primary endpoint results by analyzing the primary endpoint variable using various other methods and assumptions.

Responder analysis

Using subject responses from the modified BPI-SF3, a response variable (yes/no) will be calculated based on a reduction of at least 30% from baseline to Month 6 in the modified BPI-SF3. Subjects who have a missing modified BPI-SF3 score at Month 6 for any reason will be considered as non-responders.

The Cochran-Mantel-Haenszel (CMH) test stratified by sex and ERT treatment status at screening (treated vs not treated) will be used to test for a difference in the proportions of responders at Month 6 between treatment groups.

The treatment effect (lucerastat vs placebo) will be expressed in terms of the common odds ratio (ORc) and corresponding 95% CI. An ORc > 1 will indicate a response to treatment in favor of lucerastat as compared to placebo.

Homogeneity of the treatment effect across strata will be investigated using the Breslow-Day test.

Impact of the use of different analysis sets

An analysis on the PPS will be performed in order to assess the impact of important protocol deviations on the assessment of the primary endpoint. This analysis will especially address the issue about the lack of adherence to protocol or compliance to study treatment.

An analysis on the FAS may also be performed and will assess the impact of being randomized but not treated [see Section 10.1.8].

Impact of data collected during use of significant rescue pain therapy

The modified BPI-SF3 scores of "neuropathic pain at its worst in the last 24 hours" on the days that a subject received any significant rescue pain therapy are substituted by the worst score of that subject during the double-blind treatment period. Monthly scores are then derived as described in Section 10.2.1 and the same analysis model as described in Section 10.3.2.2 will be applied.

Characterization of treatment effect over time

The treatment effect over time will be investigated by means of a mixed model for repeated measures (MMRM) on the available monthly scores.

Use of different cut-offs for the definition of a responder at Month 6

A supportive analysis will derive the response at Month 6 by using different cut-offs for response (20%, 40%, 50%). The cut-offs of 40% and 50% have been identified in several neuropathic pain indications as substantial benefit to the subjects [Dworkin 2008]. The new response variables will then be analyzed using the CMH test as described above.

The cumulative distribution by treatment group of the percentage of subjects achieving a reduction in pain of x% at Month 6 will be presented descriptively for x ranging from 0 to 100.

Clinically important changes in modified BPI-SF3

A range of important individual subject-level changes (absolute and percentage) from baseline to Month 6 in modified BPI-SF3 will be determined using anchor-based methods [Farrar 2010, McLeod 2011]. The following four anchors will be used:

- PGIC-DS and PGIC-PS [see Section 7.2.2.5];
- PGIS-D and PGIS-P [see Section 7.2.2.4].

An analysis of the correlations between outcome (modified BPI-SF3 changes, percentage changes or absolute values at a given time point) and PGIS-P, PGIC-PS, PGIS-D and PGIC-DS will be performed in order to select the most relevant anchors to use for the determination of clinically relevant changes in modified BPI-SF3. Plots, displaying both polyserial and Spearman correlation coefficients, will be provided to assess the relationship between changes in modified BPI-SF3 and the various anchors. In order to help with the interpretation of a clinically meaningful change in modified BPI-SF3, the empirical probability density function (ePDF) and the empirical cumulative distribution function (eCDF) of the change in modified BPI-SF3 will be graphically represented for each anchor category. Adjacent anchor categories may be collapsed if the sample size within a particular anchor category is small; however, both the non-collapsed and collapsed plots will be provided. The ePDF will show the probability of observing a given BPI-SF3 change (on the y-axis) vs the change in modified BPI-SF3 (on the x-axis) and will be presented on

the same plot for each anchor category. The eCDF will show the cumulative proportion of subjects who reach a given modified BPI-SF3 change or more (on the y-axis) vs the change in modified BPI-SF3 (on the x-axis) and will be presented on the same plot for each anchor category. The eCDF plots will primarily be used to determine a reasonable range of meaningful change thresholds by estimating the 25th, 50th and 75th percentile change in modified BPI-SF3 among the improvement categories of the anchors.

In addition, supportive receiver operator characteristic curve analyses will be conducted using logistic regressions on the pre-specified response criteria defined by the anchor variables and the change in modified BPI-SF3 as explanatory variable.

Associations between change in neuropathic pain and other manifestations of FD

In order to show that improvement in neuropathic pain is not associated with deterioration of other manifestations of FD, an analysis of the correlation between the percent change from baseline in modified BPI-SF3 at Month 6 and endpoints related to GI symptoms will be provided graphically by means of a scatter plot displaying the coefficient of correlation.

10.3.2.6 Sub-group analyses

The aim of these exploratory subgroup analyses, classifying subjects according to important baseline characteristics, is to explore the consistency of treatment effect in a variety of relevant subject subgroups to support the efficacy evaluation of lucerastat in this indication.

The study is stratified by the following subgroup variables:

- Sex (male/female);
- ERT treatment status ("pseudo-naïve" / "treatment naïve" vs "switch") at screening.

The following pre-specified subgroups may also be considered for the analyses (details will be provided in the SAP):

- Geographical region (Europe, North America);
- Age (< 65 years vs ≥ 65 years);
- eGFR at screening visit (≥ 60 mL/min/1.73 m^2 vs < 60 mL/min/1.73 m^2);
- UACR at screening visit (> 30 mg/g vs ≤ 30 mg/g);
- FD complications (history of myocardial infarction, stroke, TIA, chronic kidney disease) at screening (yes/no);
- Background pain medications (none vs monotherapy vs combinations):

Monotherapy is defined as the use of pain medications belonging to one single class of pain medication. Classes of pain medication include anti-epileptics, TCAs, SNRIs, SSRIs, opioid analgesics, non-opioid analgesics [see Section 5.2.3.2];

- Background opioids (Yes/No);
- Background TCAs (Yes/No);
- Background antiepileptics (Yes/No).

Results of the subgroup analyses will be displayed in a forest plot as described in [Cuzick 2005] and will include:

1. An estimate of the treatment effect (least squares mean differences for lucerastat vs placebo) with its 95% CI for each level of each subgroup. It will be calculated using an ANCOVA as described for the primary analysis [Section 10.3.2.1]. The ANCOVA will also include the subgroup and the subgroup * treatment interaction.
2. A p-value for the interaction tests obtained from the ANCOVA described above.
3. A vertical reference line displayed at the level of the overall treatment effect.

The study is not designed or powered to detect interactions but an arbitrary two-sided significance level of alpha = 0.10 will be used for the interpretation of the interaction test. No multiplicity adjustment is introduced as the subgroup analyses are exploratory in nature.

10.3.3 Analysis of the secondary efficacy variables

10.3.3.1 Change from baseline to Month 6 in the 11-point numerical rating scale score of "abdominal pain at its worst in the last 24 hours" in subjects with gastrointestinal symptoms at baseline

This endpoint will be analyzed using the mFAS-GIS. All available data will be used regardless of occurrence of ICEs such as premature treatment discontinuation or changes in background medication.

Missing data will be imputed applying a control-based MI assuming MNAR using the CR approach, as described for the primary endpoint in Section 10.3.2.2.

An ANCOVA model will be used to analyze this endpoint on each imputed dataset. The following terms will be included in the model: baseline value, the two stratification factors (sex and ERT treatment status at screening), and the treatment group.

Uncertainty in the imputations will be reflected appropriately in the analysis by combining the results on each imputed dataset using Rubin's methodology [Rubin 1987].

The mean difference in changes from baseline to Month 6 between lucerastat and placebo together with its two-sided 95% CI and p-value will be reported.

To explore the robustness of inferences from the main analysis described above to deviations from its underlying assumptions regarding the nature of missing data, a control-based MI assuming MNAR using the J2R approach [Carpenter 2013] will be applied, as described in Section 10.3.2.4.

A supportive analysis using an MMRM approach will be conducted to characterize the treatment effect over time such that the monthly average NRS-11 scores will be analyzed on all available data from all monthly visits without replacement of missing values.

The model will enable:

- Estimation of the treatment difference (lucerastat minus placebo) in the mean changes from baseline to Month 6 along with the corresponding 95% CIs;
- Characterization of the patterns of change over time in the mean change from baseline in "abdominal pain at its worst in the last 24 hours" by treatment group.

A supportive dichotomous responder analysis will be conducted using the CMH test as described in Section 10.3.2.5. Response to study treatment on abdominal pain will be defined as a reduction from baseline to Month 6 of at least 50% in the NRS-11 score ("abdominal pain at its worst in the last 24 hours") in subjects with abdominal pain of moderate to severe intensity at baseline.

10.3.3.2 Change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale consistency Type 6 or 7 in subjects with gastrointestinal symptoms at baseline

This endpoint will be analyzed using the mFAS-GIS. All available data will be used regardless of occurrence of intercurrent events such as premature treatment discontinuation or changes in background medication. Subjects with a missing baseline value will be excluded from the analysis.

The change from baseline to Month 6 in the number of days with at least 1 stool of a Bristol Stool Scale consistency Type 6 or 7 will be analyzed using a non-parametric rank analysis of covariance [Quade 1967, Koch 1982, Koch 1990, Stokes 2012] adjusted for the baseline value and stratified by sex and ERT treatment status at screening, as follows:

1. Produce standardized ranks for the baseline variable (covariate) and the change from baseline to Month 6 variable (response) within each stratum. Standardized ranks are used to adjust for the fact that the number of subjects differ among strata.
2. Fit a separate linear regression model for each stratum with the standardized ranks of the baseline variable and the change from baseline to Month 6 variable as independent and dependent variable, respectively. Retain the regression residuals.

3. Apply the stratified Cochran-Mantel-Haenszel mean score test using the residuals as scores to compare treatment groups. The p-value from this test will be used to test the null hypothesis.

An example SAS code to perform the three steps above will be provided in the SAP.

The rank analysis of covariance does not provide an interpretable treatment effect estimate. Therefore, the magnitude of the treatment effect will be estimated using the unadjusted non-parametric win ratio [Wang 2016] between lucerastat and placebo with corresponding 95% CI. The rejection of the null hypothesis will be solely based on the p-value from the rank analysis of covariance and not the 95% CI of the unadjusted win ratio estimator.

Aligned with the treatment policy strategy, all subjects with a valid value for the change from baseline to Month 6 will be ranked based on these values (such that larger decreases are associated with better ranks). Subjects with a missing value for the change from baseline to Month 6 will be assigned worse ranks than subjects with available change from baseline to Month 6 values based on their last available change from baseline value prior to Month 6. Subjects with a non-missing baseline value but no post-baseline data will be assigned the worst rank. Mid-ranks are used in case of ties.

To explore the robustness of inferences from the main analysis described above to deviations from its assumptions regarding missing data, the above analysis will be repeated as a sensitivity analysis using the following rules for ranking of subjects with missing data. As above, subjects with a missing value for the change from baseline to Month 6 will be assigned worse ranks than subjects with available change from baseline to Month 6 values, based on their last available change from baseline value prior to Month 6. However, these subjects will then be divided into four quarters using the first quartile, median and third quartile of their last available change from baseline value. Within each quarter, lucerastat subjects will be assigned worse ranks than placebo subjects. Lucerastat subjects with a non-missing baseline value but no post-baseline data will be assigned the worst rank. Placebo subjects with a non-missing baseline value but no post-baseline data will be assigned the second worst rank. Mid-ranks are used in case of ties.

The supportive responder analysis described in Section 10.3.3.1 will also be applied to this endpoint.

10.3.3.3 Change from baseline to Month 6 in plasma globotriaosylceramide

This endpoint will be analyzed using the mFAS. All available data will be used regardless of occurrence of ICEs such as premature treatment discontinuation or changes in background medication.

Missing data will be imputed applying an MI approach assuming MAR. The imputation model includes the baseline value, the two stratification factors (sex and ERT treatment status at screening), all post-baseline monthly scores up to Month 6 and treatment group.

The MAR assumption can be considered reasonable for this endpoint as it is an objective assessment and not subject-reported and therefore less likely to be related to reasons for study withdrawal. In addition, subjects, site staff as well as the sponsor are blinded to the plasma Gb3 results until after database lock and a lower amount of missing data is expected compared to the abdominal pain and stool consistency endpoints.

After imputation, the analysis will be performed using an ANCOVA as described in Section 10.3.2.2.

A two-sided 95% CI will be calculated for the difference in the mean changes from baseline to Month 6 between lucerastat and placebo.

To explore the robustness of inferences from the main analysis described above to deviations from its underlying assumptions regarding the nature of missing data, control-based MI assuming MNAR using the CR (described in section 10.3.2.2) and J2R approaches (described in section 10.3.2.4) will be applied.

A supportive analysis using an MMRM approach will be conducted to characterize the treatment effect over time such that change in plasma Gb3 will be analyzed on all available data from all monthly visits without replacement of missing values.

The model will enable:

- Estimation of the treatment difference (lucerastat minus placebo) in the mean changes from baseline to Month 6 along with the corresponding 95% CI;
- Characterization of the patterns of change over time in the mean change from baseline in Gb3 by treatment group.

10.3.4 Analysis of other efficacy variables

The analyses of all other efficacy variables will be described in detail in the SAP. Other variables may also be analyzed by subgroups. Other efficacy endpoints will be analyzed at each relevant time point listed in the visit and assessment schedule [see Table 5 and Table 6] along with any data from unscheduled visits as appropriate.

10.3.5 Analysis of the safety variables

The SAF will be used to perform all safety analyses.

If not otherwise stated, only treatment-emergent safety data will be considered in tables and figures. All safety data will be included in listings, with flags for safety data not considered to be treatment-emergent.

10.3.5.1 Adverse events

10.3.5.1.1 Treatment-emergent adverse events and serious adverse events

Treatment-emergent AEs and SAEs will be tabulated by study treatment, system organ class (SOC) and preferred terms within each SOC: the number and percentage of subjects who experienced at least one (S)AE, at least one (S)AE within each SOC and at least one (S)AE within each preferred term will be displayed. (S)AEs will also be summarized by decreasing frequency of preferred term. (S)AEs will also be tabulated by maximum intensity and relationship to lucerastat or placebo.

10.3.5.1.2 Adverse events leading to premature discontinuation of study drug

(S)AEs leading to premature discontinuation of study drug will be summarized in a similar manner as that described in Section 10.3.5.1.1.

10.3.5.2 Vital signs

Descriptive summary statistics by visit and study treatment will be provided for observed treatment-emergent values and absolute changes from baseline in HR, SBP, DBP, MAP and body weight.

Treatment-emergent notable blood pressure abnormalities will also be summarized descriptively.

10.3.5.3 Electrocardiography

Descriptive summary statistics by visit and study treatment will be provided for observed treatment-emergent values and absolute changes from baseline in numeric 12-lead ECG values (HR, PR, QRS, QT, QTcB, and QTcF).

Changes in 12-lead ECG variables (HR, PR, QRS, QT, QTcB, and QTcF) from pre-dose to 2 h and 4 h post dose will be summarized at the Month 1 visit.

Treatment-emergent marked abnormalities for 12-lead ECG variables (HR, PR, QRS, QT, QTcB, and QTcF) will be summarized at all visits and pre/post-dose time points.

In addition, summaries of treatment-emergent morphological ECG abnormalities that were not present before first study treatment intake (using data from the ECG provider) will be provided.

10.3.5.4 Laboratory data

10.3.5.4.1 Changes from baseline in laboratory variables

Descriptive summary statistics by visit and study treatment will be provided for observed treatment-emergent values and absolute and percentage changes from baseline for laboratory tests (hematology, blood chemistry, cardiac enzyme, urinalysis).

Data will be displayed in SI units whenever possible and graphical approaches will be applied for certain variables.

10.3.5.4.2 Treatment-emergent marked laboratory abnormalities

Laboratory abnormalities will be summarized descriptively by study treatment as categorical variables.

10.3.6 Analysis of other variables

A full description of all other analyses will be described in the SAP.

10.4 Interim analyses

No interim analysis is planned for efficacy and/or futility for this study.

10.5 Sample size

The planned sample size for this study is 99 subjects using a 2:1 allocation ratio (66 lucerastat, 33 placebo) and is primarily based on a test for the mean difference between lucerastat and placebo in the change from baseline to Month 6 in the "modified" BPI-SF3 score.

The sample size calculation for the primary endpoint analysis is based on the following assumptions: a clinically relevant difference of 2 points on the 11-point scale (0–10) between lucerastat and placebo, a corresponding SD of 3 points, a two-sided type I error of 5%, and equal group variances. These assumptions come from an expected mean decrease from baseline to Month 6 in BPI-SF3 in the lucerastat group of 1.0-1.5 (2-year data for subjects on ERT; [Hoffmann 2007b]), and an expected mean increase from baseline in the placebo group of approximately 0.5 to 1.0 [Weidemann 2014]. Following these assumptions 99 subjects will provide a power of 87.2% to detect a treatment difference between lucerastat and placebo on the primary endpoint. The calculations were conducted using East 6.5 based on a two-sided t-test for independent samples.

For the analysis of the secondary efficacy endpoints, it is expected that about 70% of the randomized subjects will be included in the mFAS-GIS. Under this assumption the assessment of the secondary endpoints may be carried out on about 69 subjects (approximately 46 on lucerastat and 23 on placebo). Little is known from the literature on the expected effect size that might be observed for these endpoints however a reasonable treatment effect on the abdominal pain endpoint (NRS-11) could be a difference (delta) of

1.5–2.5 points on that scale with an expected variability (SD) in the region of 2.5–3.5. For illustration purposes, and in absence of missing data, the power to detect such an effect with this sample size for the analysis of a continuous endpoint is shown in Table 7.

Table 7 Power to detect a treatment difference for continuous secondary endpoints analyzed on the mFAS-GIS for a range of deltas and SDs (n = 69 subjects)

alpha = 5% two-sided			
delta	SD = 2.5	SD = 3.0	SD = 3.5
1.5	63.9%	48.8%	38.0%
2.0	87.0%	73.0%	59.7%
2.5	97.1%	89.5%	78.7%

SD = standard deviation.

11 DATA HANDLING

11.1 Data collection, data transfer procedure and data access

The investigator/delegate is responsible for ensuring the accuracy, completeness and on time reporting of subject data. All source documents should be completed in a neat, legible manner to ensure accurate interpretation and traceability of the data. Data reported in the eCRF derived from source documents must be consistent with the source documents.

eCRF data will be captured via Electronic Data Capture. The investigator and site personnel will be trained to enter and edit the data via a secure network, with secure access features (username, password, and identification — an electronic password system). A complete electronic audit trail will be maintained. The investigator/delegate will approve the data (i.e., confirm the accuracy of the data recorded) using an electronic signature (ref. to US 21 CFR Part 11).

Entries recorded by the subject in the eDiary are considered source data. All records on the eDiary will be date and time stamped. Each time the subject makes an entry into the eDiary, he/she will be required to enter his/her own confidential password. The eDiary device will transfer data to the eDiary vendor's central server automatically on a nightly basis. The communication software ensures a secure transfer of data by encryption. The data will remain in an encrypted state during transmission to the server. The transfer process is validated to ensure that the data on the central server is an accurate and complete copy of the eDiary data. Once the data are on the central server, the site staff, eDiary vendor, and sponsor representatives can access the data in a view-only mode on a 24 hours per day, 7 days per week basis. This data access is role-based and strictly view-only. Access to the data on the server requires a valid user identification and password combination. Site personnel will review and ensure completeness of the subjects' entries.

For each subject enrolled, regardless of study treatment initiation, an eCRF must be completed and signed by the investigator/delegate. This also applies to those subjects who fail to complete the study.

11.2 Maintenance of data confidentiality

The investigator/delegate must ensure that data confidentiality is maintained. On eCRFs or other documents (e.g., documents attached to SAE forms / Pregnancy forms) submitted to the sponsor and any vendors or CROs, subjects must be identified only by number and never by their name or initials, date of birth, hospital numbers, or any other personal identifier. An exception will be made for the vendor in charge of the exit interviews who will obtain subject's name and contact details from the investigator/delegate. The investigator/delegate must keep a subject identification code list at the site, showing the screening/randomization number, the subject's name, date of birth, and address or any other locally accepted identifiers. Documents identifying the subjects (e.g., signed ICFs) must not be sent to the sponsor, any vendor or CROs, and must be kept in strict confidence by the investigator/delegate.

In the eDiary, there will be no possibility to enter any personal identifier and the subject will only be identified by the study site and subject number. The eDiary device will be authenticated when it attempts to transfer data to the vendor's central server; the authentication will be based upon subject identification (i.e., site and subject number) pre-loaded in the vendor's server. The server will verify both the serial number of the eDiary device and the subject identifier before any data can be transferred into the system.

11.3 Database management

eCRFs will be used for all subjects. The investigators will have access to the site eCRF data until the database is locked. The eCRF must be kept current to reflect subject status at any time point during the course of the study.

While entering the data, the investigator/delegate will be instantly alerted to data queries by validated programmed checks. Additional data review will be performed by the sponsor personnel on an ongoing basis to look for unexpected patterns in data and for study monitoring. Should discrepant data be detected, a query specifying the matter and requesting clarification will be issued and visible to the investigator/delegate via the eCRF. All electronic queries visible in the system either require a data correction (when applicable) and a response from the investigator/delegate to clarify the queried data directly in the eCRF, or simply a data correction in the eCRF. The investigator/delegate must, on request, supply the sponsor with any required background data from the study documentation or clinical records. This is particularly important when errors in data transcription are suspected. In the case of health authority queries, it is also necessary to have access to the complete study records, provided that subject confidentiality is protected.

This process will continue until database lock.

The test results of laboratory samples, ECGs, echocardiographies, eDiary, processed through a central laboratory or vendor and the results of the randomized subjects will be electronically sent to the sponsor at pre-specified intervals with a final transfer prior to database lock. During the course of the study, the site staff and sponsor representatives can access the data in a view-only mode on the central server of the respective vendor [see also Section 11.1].

AEs and medical history are coded according to the latest MedDRA™ version used by the sponsor or its delegate. Medications are coded according to the latest WHODrug Dictionary version used by the sponsor or its delegate.

After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time may only be made as described in the appropriate sponsor QS docs. After database lock, the investigator will have read-only access to the site eCRF subject data, until receipt of an electronic copy of the site eCRFs (including the audit trail) on electronic media.

12 PROCEDURES AND GOOD CLINICAL PRACTICE

12.1 Ethics and Good Clinical Practice

The sponsor personnel and the investigators will ensure that the study is conducted in full compliance with ICH GCP guidelines, the principles of the "Declaration of Helsinki", and with the laws and regulations of the country in which the study is conducted.

12.2 Independent Ethics Committee / Institutional Review Board

The investigator will submit this protocol and any related document(s) provided to the subject (such as the ICF) to an IEC/IRB. Approval from the committee/board must be obtained before starting the study and must be documented in a dated letter to the investigator, clearly identifying the study, the documents reviewed, and the date of approval.

Modifications made to the protocol or the ICF after receipt of the approval must also be submitted as amendments by the investigator to the IEC/IRB in accordance with local procedures and regulations [see Section 12.6].

A list of members participating in the IEC/IRB meetings must be provided, including the names, qualifications, and functions of these members. If that is not possible, the attempts made to obtain this information along with an explanation as to why it cannot be obtained or disclosed must be documented in the study documentation.

If a member of the site study personnel was present during an IEC/IRB meeting, it must be clear that this person did not vote.

12.3 Informed consent

It is the responsibility of the investigator/delegate to obtain informed consent according to ICH GCP and Declaration of Helsinki guidelines and local regulations from each individual subject participating in this study and/or his/her legally designated representative. The investigator/delegate must explain to subjects that they are completely free to refuse to enter the study, or to voluntarily withdraw from the study at any time for any reason without having to provide any justification. Special attention shall be paid to the information needs of specific subject populations and of individual subjects, as well as to the methods used to give the information. Adequate time shall be given for the subject to consider his or her decision to participate in the study and it shall be verified that the subject has understood the information (e.g., by asking the subject to explain what is going to happen). In the US and Canada, the ICF will include a description of the exit interview component of the study.

The ICF will be provided in the country local language(s).

Site personnel authorized (according to local regulation) to participate in the consent process and/or to obtain consent from the subject will be listed on the Delegation of Authority form.

The subject and authorized site personnel listed on the Delegation of Authority form must sign, personally date, and time (if the first study-mandated procedure is to be performed on the same day informed consent is obtained) the ICF before any study-related procedures (i.e., any procedures required by the protocol) begin.

If pregnancy of a male participant's female partner is reported as defined in Section 9.4.1, she will be asked to sign a specific ICF allowing the collection, storage and use of data about the pregnancy and the birth of the child. The ICF will be provided in the country local language(s).

12.4 Compensation to subjects and investigators

The sponsor provides insurance in order to indemnify (with both legal and financial coverage) the investigator/site against claims arising from the study, except for claims that arise from malpractice and/or negligence.

The compensation of the subject in the event of study-related injuries will comply with applicable regulations.

12.5 Protocol adherence/compliance

The investigator must conduct the study in compliance with the IEC/IRB and/or the regulatory authority-approved version of the protocol and must not implement any deviation/change from the protocol, except when deviation is necessary to eliminate an immediate hazard to the subject.

If a protocol deviation occurs, the investigator/delegate will inform the sponsor or its representative in a timely manner. The investigator/delegate must document and explain any deviation from the approved protocol. Deviations considered to be a violation of ICH GCP must be reported to the IEC/IRB and regulatory authorities according to the sponsor or (overruling) local requirements.

All protocol deviations will be reported in the CSR. IECs/IRBs will be provided with listings of protocol deviations as per local requirements.

12.6 Protocol amendments

Any change to the protocol can only be made through a written protocol amendment. An amended protocol must be submitted to the IEC/IRB and regulatory authorities, according to their requirements.

12.7 Essential documents and retention of documents

The investigator/delegate must maintain adequate records necessary for the reconstruction and evaluation of the study. A number of attributes are considered of universal importance to source data and the records that hold those data. These include that the data and records are accurate, legible, contemporaneous, original (or certified copy), attributable, complete, consistent, enduring, and available when needed.

These records are to be classified into 2 different categories of documents: ISF and subjects' source documents.

These records must be kept by the investigator for as long as is necessary to comply with the sponsor's requirements (i.e., as specified in the clinical study agreement), and national and/or international regulations, whichever would be the longest period. If the investigator cannot guarantee this archiving requirement at the site for any or all of the documents, special arrangements, respecting the data confidentiality, must be made between the investigator and the sponsor to store these documents outside the site, so that they can be retrieved in the event of a regulatory inspection. No study document should be destroyed without prior written approval from the sponsor. Should the investigator wish to assign the study records to another party, or move them to another location, the sponsor must be notified in advance.

If the site is using an electronic/computerized system to store subject medical records, it can be used for the purpose of the clinical study if it is validated (as per 21 CFR Part 11 or equivalent standard) and if the CRA has been provided personal and restricted access to study subjects only, to verify consistency between electronic source data and the eCRF during monitoring visits.

If the site is using an electronic/computerized system to store subject medical records but it could not be confirmed that the system is validated or if the CRA could not be provided

access to the system, the site is requested to print the complete set of source data needed for verification by the CRA. The print-outs must be numbered, stapled together with a coversheet, signed and dated by the investigator/delegate to confirm that these certified copies are exact copies having the same information as the original source data. The printouts will be considered as the official clinical study records and must be filed either with the subject's medical records or with the subject's eCRF.

In order to verify that the process the site uses to prepare certified copies is reliable, the CRA must be able to observe this process and confirm that the comparison of the source documents and the certified copy did not reveal inconsistencies. The CRA does not need to verify this process for all data of all subjects but at least for some of them (e.g., first subject; regular check during the study of critical data like inclusion/exclusion criteria, endpoints for some subjects) as per the sponsor instructions. If it were not possible for the CRA to observe this process, it would not be possible to rely on the site's certified copies and therefore the site cannot be selected for the clinical study.

12.8 Monitoring

Prior to study start, at a site, all required approvals must be obtained. A site initiation visit (SIV) will be performed after the required essential study documents are approved by the sponsor. The study treatment will be shipped to the site upon approval of the required essential documents.

The PI must ensure that all site personnel involved in the study are present during the SIV and will dedicate enough time to it. Site Information Technology support should also be available during the SIV to assess the use of the eCRF.

The SIV must be completed before the site can start the screening of study subjects. Following the SIV, a copy of the completed initiation visit report and follow-up letter will be provided to the PI and filed in the ISF.

During the study, the CRA will contact and visit the site regularly and must be permitted, on request, to have access to study facilities and all source documents needed to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered in the eCRFs and other protocol-related documents. The sponsor monitoring standards require full verification that informed consent has been provided, verification of adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of the main efficacy, safety, and tolerability endpoints. Additional checks of the consistency of the source data with the eCRFs will be performed according to the study-specific monitoring guidelines. The frequency of the monitoring visits will be based on subject recruitment rate and critical data-collection times.

The PI must ensure that the eCRF is completed after a subject's visit (site visit or telephone call), and that all requested subject files (e.g., ICFs, medical notes/charts, other

documentation verifying the activities conducted for the study) are available for review by the CRA. The required site personnel must be available during monitoring visits and allow adequate time to meet with the CRA to discuss study-related issues.

The investigator agrees to cooperate with the CRA(s) to ensure that any issues detected in the course of these monitoring visits are resolved. If the subject is hospitalized or dies in a hospital other than the study site, the investigator is responsible for contacting that hospital in order to document the SAE, in accordance with local regulations.

A close-out visit will be performed for any initiated site when there are no more active subjects and all follow-up issues have been resolved. If a site does not enroll any subjects, the close-out visit may be performed prior to study closure at the discretion of the sponsor.

12.9 Investigator Site File

Each site will be provided with an ISF prior to the SIV. It will contain all the essential documents that are required to be up-to-date and filed at site as per ICH GCP section 8.

The ISF will include a table of content listing the essential documents. All study-related documentation must be maintained in the ISF.

In some cases, exceptions can be discussed with the CRA regarding the filing of the study documents outside the ISF. It should be clearly documented where each document is filed. This note to file should be present in the specific tab of the document in the ISF.

The ISF must be stored in a secure and access-restricted area during and after the study. The ISF must be kept by the site for as long as is necessary to comply with the sponsor's requirements (i.e., as specified in the clinical study agreement), ICH GCP and national and/or international regulations, whichever would be the longest period. If the site needs to transfer the ISF to another location and/or if site facility can no longer store the ISF, the PI must immediately inform the sponsor.

If the PI will change, or if the site will relocate, the CRA must be notified as soon as possible.

12.10 Audit

The sponsor representatives may audit the investigator site (during the study or after its completion). The purpose of this visit will be to determine the investigator's adherence to ICH GCP, the protocol, and applicable regulations; adherence to the sponsor requirements (e.g., standard operating procedures) will also be verified. Prior to initiating this audit, the investigator will be contacted by the sponsor to arrange a time for the audit.

The investigator and site personnel must cooperate with the auditor(s) and allow access to all study documentation (e.g., subject records) and facilities.

12.11 Inspections

Health authorities and/or IEC/IRB may also conduct an inspection of this study (during the study or after its completion) at the site.

Should an inspection be announced by a health authority and/or IEC/IRB, the investigator must immediately inform the sponsor (usually via the CRA) that such a request has been made.

The investigator and site personnel must cooperate with inspector(s) and allow access to all study documentation (e.g., subject records) and study facilities.

12.12 Reporting of study results and publication

The sponsor will post the key elements of this protocol and the summary of results within the required timelines on publicly accessible databases (e.g., clinicaltrials.gov, EU database), as required by law and regulation.

Study results will be documented in a CSR that will be signed by the sponsor's representatives and the coordinating investigator.

In accordance with the Good Publication Practices and ethical practice as outlined in internationally recognized guidance documents (e.g., European Medical Writers Association, American Medical Writers Association, International Society for Medical Publication Professionals), the results of the study will be submitted for publication in a peer-reviewed journal. Study results can be submitted for presentation at a congress before submission to a peer-reviewed journal.

The coordinating investigator and the Steering Committee, if any, will have the opportunity to review the analysis of the data and to discuss the interpretation of the study results with the sponsor personnel prior to submission to a peer-reviewed journal or presentation at a congress.

Authorship will be determined in accordance with the International Committee of Medical Journal Editors criteria, and be based on:

- substantial contributions to the conception or design of the study, or the acquisition, analysis, or interpretation of data; and
- drafting of the publication or critical review for important intellectual content; and
- providing final approval of the version to be published; and
- agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

The list of authors of any publication of study results may include representatives of the sponsor and will be determined by mutual agreement.

Any study-related publication written independently by investigators must be submitted to the sponsor for review at least 30 days prior to submission for publication or presentation at a congress. Upon review, the sponsor may provide comments, and may also request alterations and/or deletions for the sole purpose of protecting its confidential information and/or patent rights. Neither the institution nor the investigator should permit publication during such a review period.

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14 APPENDICES

Appendix 1 Individualized Pain Management Plan template

Subject name:		IPMP version:	
Subject number:		Date:	

Study Doctor	Study Nurse / Coordinator
Name: Medical institution: Address / E-mail: Telephone:	Name: Medical institution: Address / E-mail: Telephone:

In order to help you manage your pain, it is important that you continue taking the following medications as prescribed by your study doctor:

Pain medication (name)	Dose	Frequency	Additional instructions

If you suffer from unbearable pain despite using the medication(s) listed above, it is important to follow the steps below:

1. Contact your doctor/study nurse as soon as possible. Note that your doctor/study nurse may invite you to go back to the hospital.
2. Take the rescue pain medication according to the instructions listed below; you may need to get the rescue pain medication using the prescription provided to you by the doctor/study nurse.
3. In the evening, complete your electronic diary as usual. Remember to enter the use of rescue pain medication in the eDiary.
4. After 72 hours, your doctor/study nurse will contact you to check your pain status. Note that your doctor/study nurse may invite you to go back to the hospital to discuss alternative treatments.

Rescue pain medication (name)	Dose	Frequency	Additional instructions

I, the undersigned, have been informed of the purpose of the Individual Pain Management Plan and I acknowledge I fully understand the instructions on how to apply it. I am aware that I will receive a copy of this signed Individual Pain Management Plan and that the information will be entered in my eDiary as well.

Study patient:				
	Print Name	Signature	Date	Time

I, the undersigned, have informed the study participant named above about the purpose and the relevant details to implement this Individual Pain Management.

Study or delegate:				
	Print Name	Signature	Date	Time

Reminder: A copy of this IPMP must be given to the participant.

Instructions for the investigator/delegate (not to be given to the patient)

During the screening visit:

1. Discuss with your patient the possibility to use pain medications during the study.
2. Complete the IPMP version 1:
    a. Indicate which pain medication the subject is currently taking and should continue taking.
    b. Indicate which rescue pain medication should be used by the subject if they are experiencing unbearable pain (see list below). Take into account the patient's background pain medications and his/her previous response to pain medication.
3. Instruct the patient to continue taking his/her pain medication(s) and, in the case of unbearable pain, to follow the rescue pain medication plan listed in the IPMP.
4. Instruct the patient to contact the study nurse or the study doctor, in the case of unbearable pain. Ensure the contact details for nurse and physician are completed in the IPMP.
5. Ensure the patient got the prescription needed to obtain pain medication (including rescue pain medication) listed in the IPMP.
6. Both patient and investigator must sign the IPMP and a copy should be provided to the patient.

During the randomization visit and any subsequent study visits:

1. Check whether patient is compliant with the IPMP and eDiary completion.
2. Check whether patient considers his/her pain to be adequately managed.
3. If needed, update the IPMP (e.g., IPMP version 2).
4. Ensure the patient got the prescription needed to obtain pain medication (including rescue pain medication) listed in the IPMP.
5. Both patient and investigator must sign the updated IPMP version and a copy should be provided to the patient.

In the event of of subject phone call due to unbearable pain:

1. Check if the patient has continued to take his/her pain medication as listed in the IPMP.

2. Ask the patient if he/she has completed the eDiary.
3. Remind the patient of the rescue pain medication, of what he/she should take as per IPMP. Remind the patient to enter the use of all pain medications in the eDiary.
4. Schedule a phone call with the patient, 72 hours after this phone call.

72-hour phone call:

1. Ask the patient whether the pain symptoms have improved, worsened or remained stable.
2. Check whether the eDiary was completed (pain intensity and use of pain medications).
3. Ask the patient if he/she considered is pain is adequately managed with the rescue pain medication. If not, advise the patient if any adjustment on pain medication dose is required. You may also consider scheduling a visit to the site to update the IPMP (e.g., adding a new rescue pain medication to the IPMP).

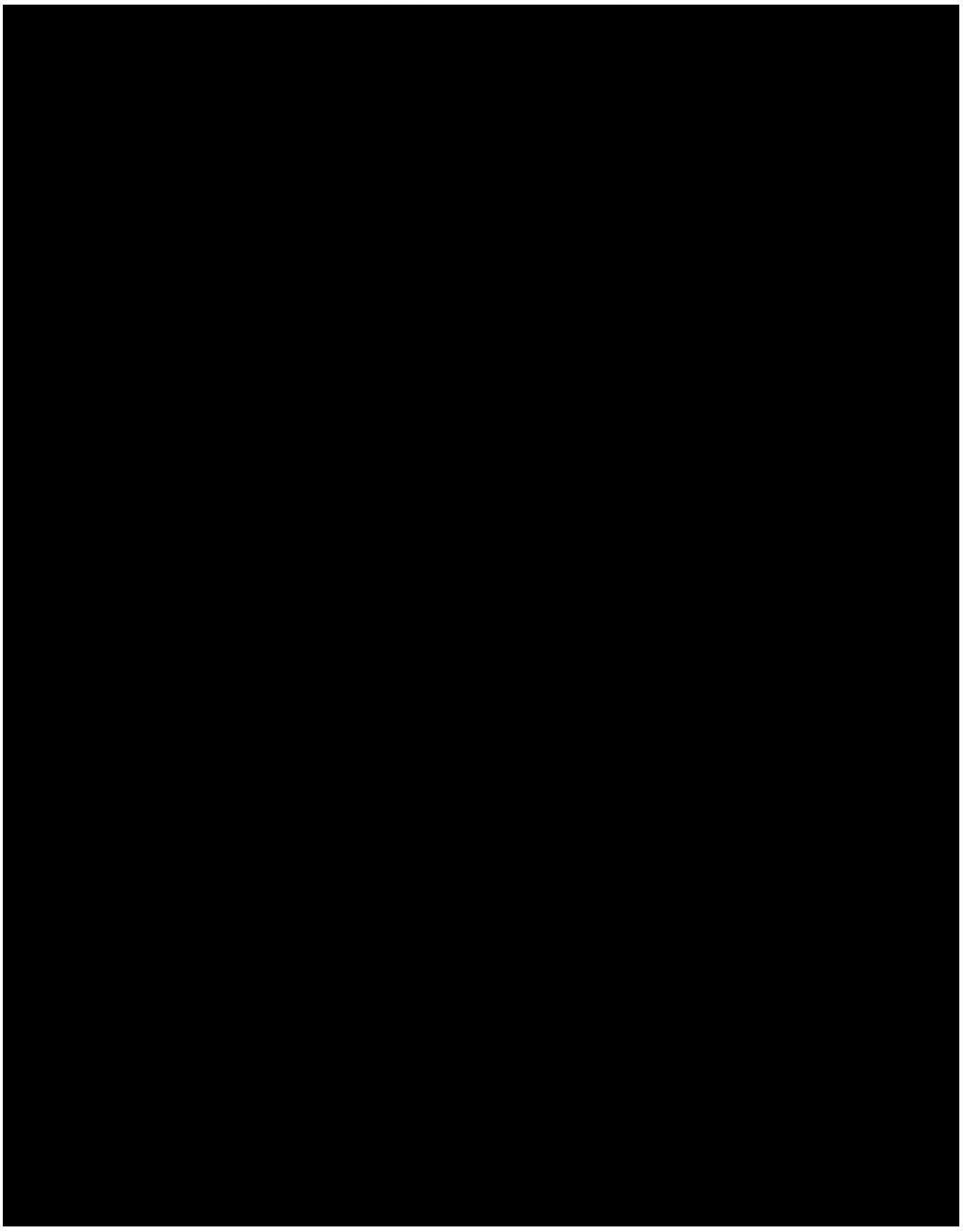
During unscheduled site visit due to unbearable pain:

1. Review the IPMP with the patient; discuss additional rescue pain medications.
2. If needed, update the IPMP (IPMP version 2) by adding/replacing or increasing the dose of rescue pain medications.
3. Ensure the patient got the prescription needed to obtain pain medication (including rescue pain medication) listed in the IPMP.
4. Both patient and investigator must sign the updated IPMP version and a copy should be provided to the patient.

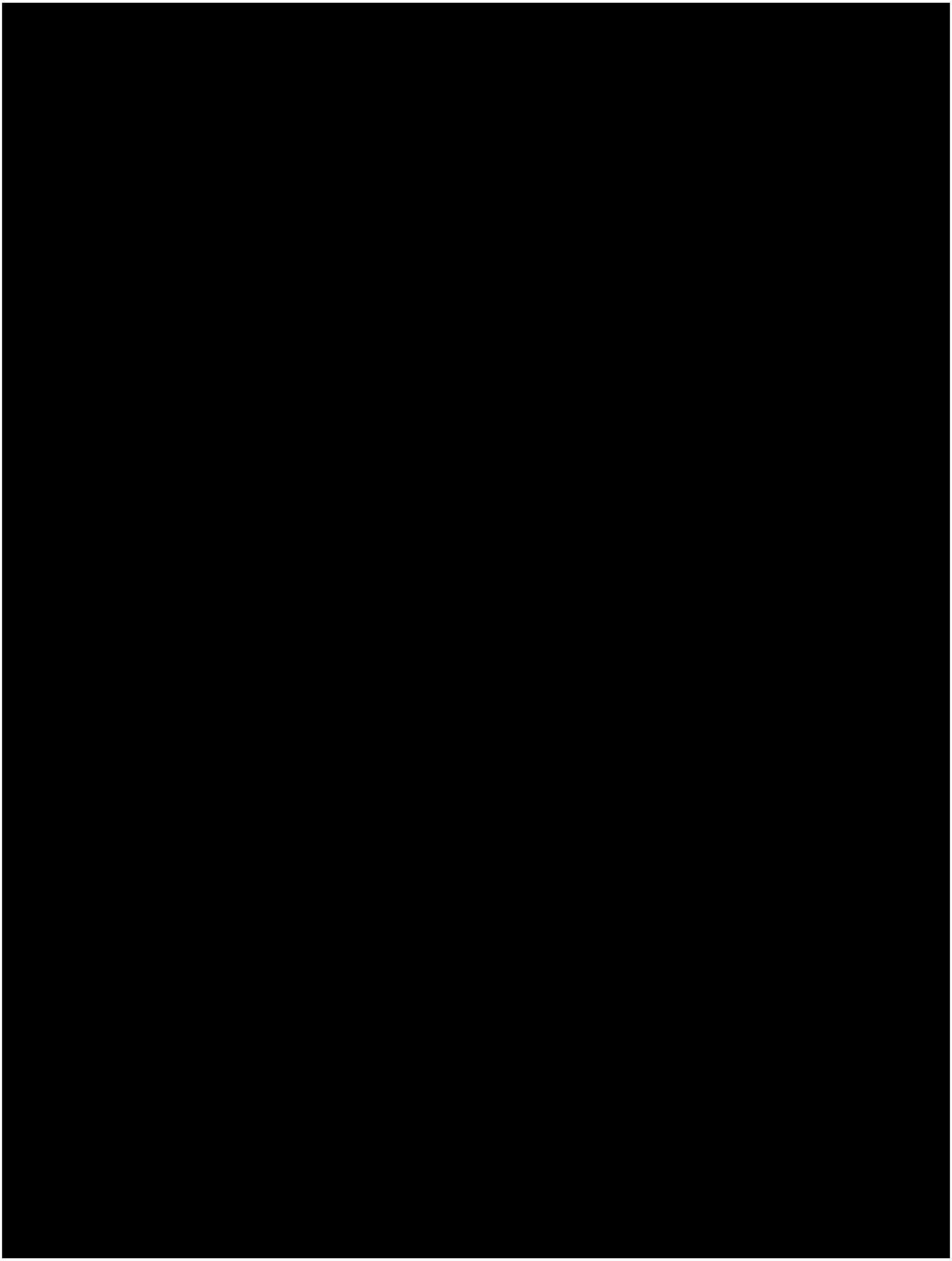
Example of potential rescue therapy for unbearable pain [see protocol section 5.2.3.2.2]:

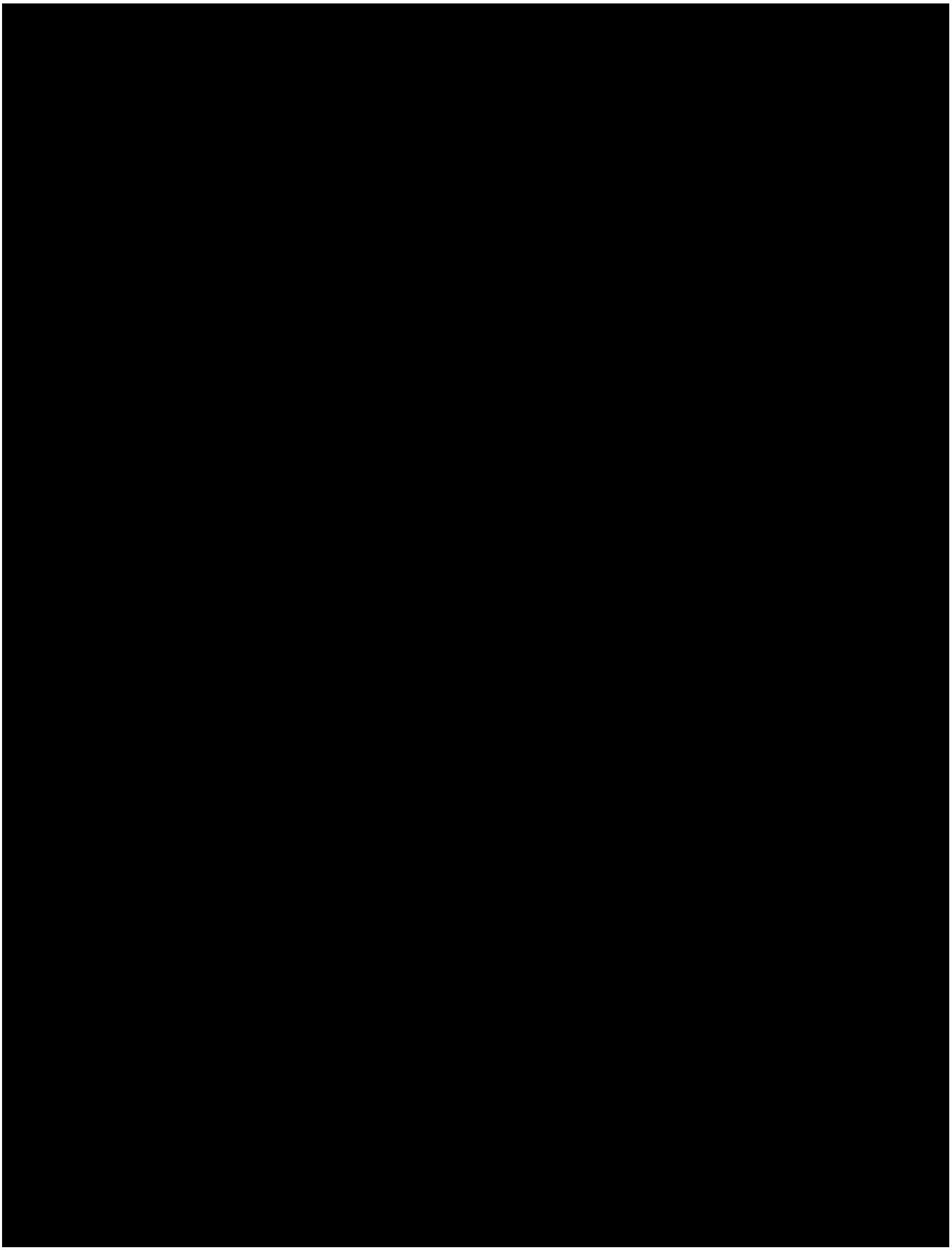
- Initiation or dose escalation of adjuvant pain medication (i.e., anti-epileptics, tricyclic antidepressants, antidepressants belonging to SNRIs/SSRIs);
- Initiation or dose escalation of opioid analgesic drugs;
- Initiation or dose escalation of non-opioid analgesics (i.e., NSAIDs, topical analgesics).

Appendix 2 Bristol Stool Scale



Appendix 3 Brief Pain Inventory-Short Form – English version (24 h)





Appendix 4 Patient Global Impression questionnaires

Patient Global Impression of Severity of neuropathic Pain (PGIS-P)

The following question refers to the neuropathic pain you may experience due to your Fabry disease.

Please choose the response that best describes the overall severity of the neuropathic pain you may have experienced due to your Fabry disease over the past 7 days (week):

- 1=None
- 2=Mild
- 3=Moderate
- 4=Severe

Patient Global Impression of Change in neuropathic Pain Severity (PGIC-PS)

The following question refers to the neuropathic pain you may experience due to your Fabry disease.

Please choose the response that best describes the change in the overall severity of the neuropathic pain you may have experienced due to your Fabry disease since you started study treatment:

- 1 = Very Much Improved
- 2 = Much Improved
- 3 = Minimally Improved
- 4 = No change
- 5 = Minimally worse
- 6 = Much Worse
- 7 = Very Much Worse

Patient Global Impression of Severity of Disease (PGIS-D)

The following question refers to symptoms (e.g., neuropathic pain, abdominal pain, diarrhea, constipation) you may experience due to your Fabry disease.

Please choose the response that best describes the overall severity of the symptoms you may have experienced due to your Fabry disease over the past 7 days (week):

- 1=None
- 2=Mild
- 3=Moderate
- 4=Severe

Patient Global Impression of Change in Disease Severity (PGIC-DS)

The following question refers to symptoms (e.g., neuropathic pain, abdominal pain, diarrhea, constipation) you may experience due to your Fabry disease.

Please choose the response that best describes the change in overall severity of the symptoms you may have experienced due to your Fabry disease since you started study treatment:

- 1 = Very Much Improved
- 2 = Much Improved
- 3 = Minimally Improved
- 4 = No change
- 5 = Minimally worse
- 6 = Much Worse
- 7 = Very Much Worse

Appendix 5 Center for Epidemiologic Studies Depression Scale Revised

Center for Epidemiologic Studies Depression Scale – Revised (CESD-R)

Below is a list of the ways you might have felt or behaved. Please check the boxes to tell me how often you have felt this way in the past week or so.	Last Week				Nearly every day for 2 weeks
	Not at all *or* Less than 1 day	1 - 2 days	3 - 4 days	5 - 7 days	
My appetite was poor.	0	1	2	3	4
I could not shake off the blues.	0	1	2	3	4
I had trouble keeping my mind on what I was doing.	0	1	2	3	4
I felt depressed.	0	1	2	3	4
My sleep was restless.	0	1	2	3	4
I felt sad.	0	1	2	3	4
I could not get going.	0	1	2	3	4
Nothing made me happy.	0	1	2	3	4
I felt like a bad person.	0	1	2	3	4
I lost interest in my usual activities.	0	1	2	3	4
I slept much more than usual.	0	1	2	3	4
I felt like I was moving too slowly.	0	1	2	3	4
I felt fidgety.	0	1	2	3	4
I wished I were dead.	0	1	2	3	4
I wanted to hurt myself.	0	1	2	3	4
I was tired all the time.	0	1	2	3	4
I did not like myself.	0	1	2	3	4
I lost a lot of weight without trying to.	0	1	2	3	4
I had a lot of trouble getting to sleep.	0	1	2	3	4
I could not focus on the important things.	0	1	2	3	4

REFERENCE: Eaton, W. W., Smith, C., Ybarra, M., Muntaner, C., Tien, A. (2004). Center for Epidemiologic Studies Depression Scale: review and revision (CESD and CESD-R). In ME Maruish (Ed.). *The Use of Psychological Testing for Treatment Planning and Outcomes Assessment* (3rd Ed.), Volume 3: Instruments for Adults, pp. 363-377. Mahwah, NJ: Lawrence Erlbaum.

Appendix 6 36-Item Short Form Health Survey Version 2