STATISTICAL ANALYSIS PLAN FOR ID-069A301 CLINICAL STUDY REPORT

MODIFY

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

α-GalA	α-galactosidase A
AE	Adverse event
ANCOVA	Analysis of covariance
ARRT	Annualized rate of days with significant rescue therapy
AUC_τ	Area under the plasma concentration-time curve during one dosing interval
BLQ	Below the limit of quantification
BPI-SF	Brief Pain Inventory-Short Form
BPI-SF3	Brief Pain Inventory-Short Form item 3
bpm	Beats per minute
BSA	Body surface area
BSS	Bristol Stool Scale
CDISC	Clinical Data Interchange Standards Consortium
CESD-R-20	Center for Epidemiologic Studies Depression revised scale
CI	Confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
C_{max}	Maximum plasma concentration
CMH	Cochran-Mantel-Haenszel
COVID-19	Coronavirus disease 2019
CR	Copy Reference
CV	Coefficient of variation
DBP	Diastolic blood pressure
DSM	Diagnostic and Statistical Manual of Mental Disorders
eCDF	Empirical cumulative distribution function
ECG	Electrocardiogram
eCRF	Electronic case report form
eDiary	Electronic diary
eGFR	Estimated glomerular filtration rate
EOS	End-of-Study
EOT	End-of-Treatment
ePDF	Empirical probability density function

ERT	Enzyme replacement therapy
FAS	Full analysis set
FD	Fabry disease (Anderson-Fabry disease)
FDA	US Food and Drug Administration
FU	Follow-up
Gb3	Globotriaosylceramide
GI	Gastrointestinal
GlcCer	Glucosylceramide (Gb1)
HR	Heart rate
ICE	Intercurrent event
IRT	Interactive Response Technology
J2R	Jump to Reference
λ_z	First order rate constant associated with the terminal log-linear portion of the plasma concentration-time curve
LacCer	Lactosylceramide (Gb2)
LVMI	Mass-I left ventricle
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
OLE	Open-label extension
OR	Odds ratio
ORc	Common odds ratio
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	Patient Global Impression of Severity
PGIS-D	Patient Global Impression of Severity of Disease
PGIS-P	Patient Global Impression of Severity of neuropathic Pain
PK	Pharmacokinetic(s)
PPS	Per-protocol analysis set
PTOP	Post-treatment observation period
QTc	Corrected QT interval
QTcB	QT corrected according to Bazett's formula
QTcF	QT corrected according to Fridericia's formula
ROC	Receiver operating characteristic
SAE	Serious adverse event
SAF	Safety set
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SCR	Screened analysis set
SD	Standard deviation
SE	Standard error
SI	Standard international
SNRI	Serotonin-norepinephrine re-uptake inhibitor
SOC	System organ class
SOP	Standard Operating Procedures
SSRI	Selective serotonin re-uptake inhibitor
$t_{½}$	Apparent terminal elimination half-life
TCA	Tricyclic antidepressant
TEAE	Treatment-emergent adverse event
t_{max}	Time to reach maximum plasma concentration
UACR	Urine albumin-to-creatinine ratio
US	United States

1 INTRODUCTION

This statistical analysis plan (SAP), based on global protocol version 5 of 24 August 2021 (and local protocol version 5.VHP.B of 25 August 2021), describes in detail the analyses and presentation for the clinical study report for ID-069A301 (MODIFY) of the primary, secondary and exploratory efficacy endpoints as well as all safety endpoints, quality of life endpoints, pharmacokinetic (PK) endpoints, and biomarker endpoints.

This SAP does not cover the analysis of the exit interview sub-study.

Source data for the analyses will be provided as SAS® datasets according to the Clinical Data Interchange Standards Consortium (CDISC) Study Data Tabulation Model. Analysis datasets will be derived as SAS® datasets according to the CDISC Analysis Data Model.

The Clinical Pharmacologist performs the calculation of PK parameters and creates figures of individual and mean concentration-time profiles. PK concentration data will be merged with the actual date/time of sampling by Idorsia Data Management.

2 STUDY DESIGN AND FLOW

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

118 adult subjects with Fabry disease (FD) exhibiting Fabry-associated pain of moderate to severe intensity were randomized in a 2:1 ratio to either lucerastat (approximately 79 subjects) or placebo (approximately 39 subjects).

Treatment allocation was stratified by

- sex

and

- enzyme replacement therapy (ERT) treatment status at screening
  - "pseudo-naïve" / "treatment naïve" (not treated in last 6 months / never treated)

  vs
  - "switch" (subjects treated with ERT at screening).

Once randomized, subjects entered a 6-month, double-blind treatment period. Approximately 36 subjects were included worldwide in a PK profile sub-study.

All English-speaking subjects in the US and Canada were asked to participate in an exit interview sub-study; subjects who accepted performed this interview as soon as possible after the Month 6 or End-of-Treatment (EOT) visit, ideally within 2 weeks.

No interim analysis was planned.

2.1 Study periods

The study comprises the following consecutive periods as described in section 3.1.1 of the protocol:

Screening period: Lasts approximately 6–7 weeks; starts with the signing of the informed consent form (screening visit) and ends with the day before subject randomization. During this period, the subjects will start completing an electronic diary (eDiary) on a daily basis.

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

The EOT visit will take place at Month 6 (or earlier in the event of premature treatment 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 the PTOP which starts on the day after the last dose of study treatment and ends at the 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.

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 offered enrollment 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 / Institutional Review Boards).

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.

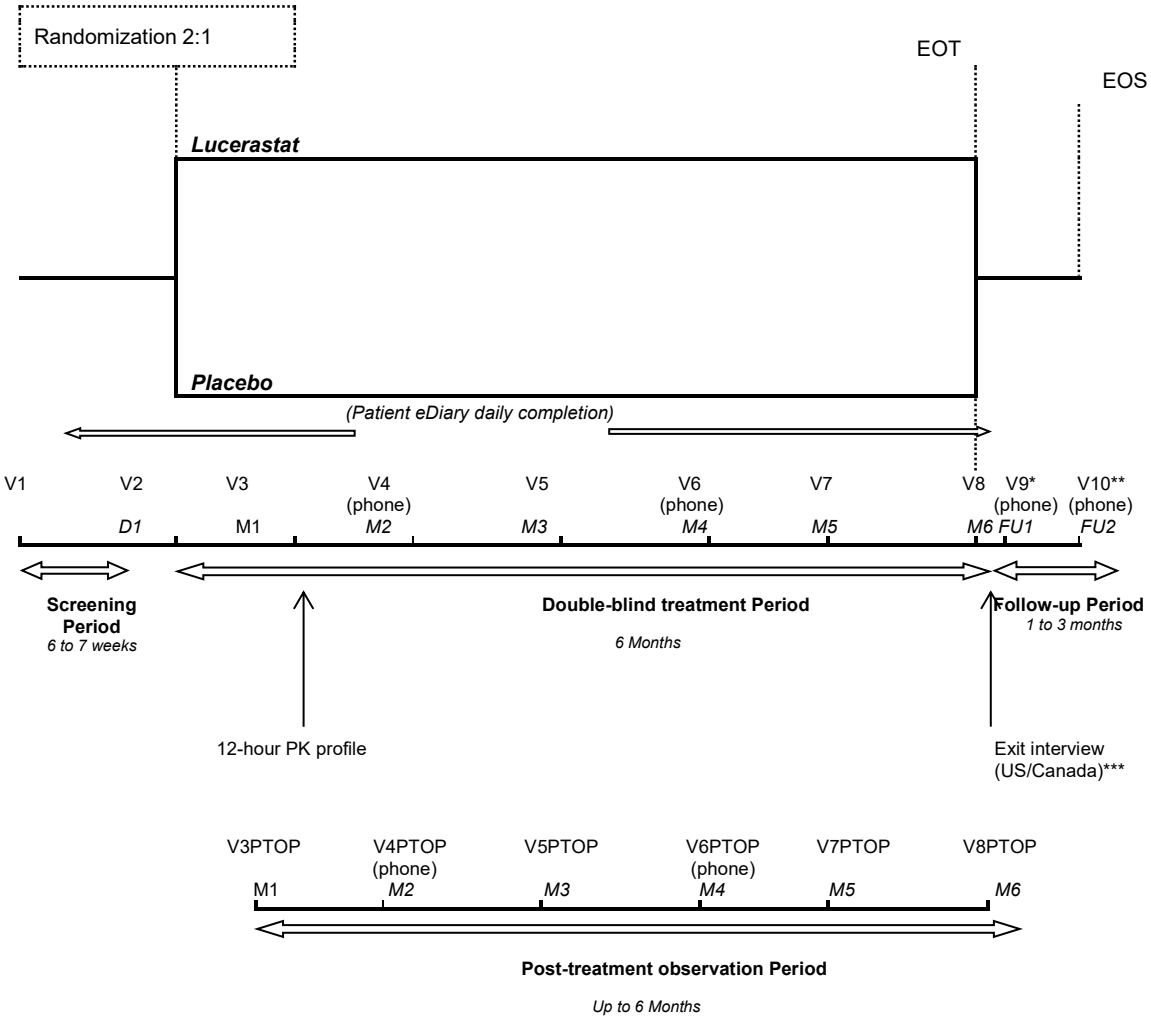
For an individual subject, the End-of-Study (EOS) is defined as follows:

- 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, the 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 table 4 and table 5 of the protocol and are described in protocol 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; eDiary = electronic diary; EOT = End-of-Treatment; EOS = End-of-Study; FU = follow-up (telephone); M = month; OLE = open-label extension; PK = pharmacokinetic(s); PTOP = post-treatment observation period; V = visit.

2.2 Study duration and global end of 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 participation in the study of a subject is expected to be about 9 months for a female subject and about either 9 or 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 OBJECTIVES

3.1 Primary objective

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

3.2 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.

3.3 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 quality of life in subjects with FD.
- To document the PK of lucerastat in subjects with FD.

3.4 Efficacy estimands

The estimands targeted by the primary and secondary efficacy objectives and analyses are defined in Table 1.

A treatment policy strategy will be applied for all estimands defined in Table 1, 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.

Fabry disease medications (e.g., ERT) are forbidden by the protocol as they are not part of the treatment condition of interest [see definition in Table 1]. 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 1 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 the study protocol.	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 the study protocol.	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 the study protocol 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 the study protocol 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.

4 ANALYSIS SETS

4.1 Definitions of analysis sets

4.1.1 Screened analysis set

The Screened analysis set (SCR) includes all subjects who are screened and have a subject identification number.

4.1.2 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:

- Subjects will be evaluated according to their assigned study treatment (not actual treatment received) and stratum information as recorded in the Interactive Response Technology (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.

4.1.3 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.

4.1.4 Modified full analysis set – gastrointestinal symptoms

The mFAS-GI 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 11-point numerical rating scale (NRS-11) scale; and/or
- diarrhea at baseline defined as having at least 1 stool of Bristol Stool Scale (BSS) consistency Type 6 or 7 on at least 8 days.

4.1.5 Per-protocol analysis set

The Per-protocol analysis set (PPS) comprises 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 a separate document that will be finalized and stored in the eTMF before breaking the randomization blind.

4.1.6 Safety set

The Safety set (SAF) includes all subjects who received at least one 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 treatments to the same patient, the patient will be counted as on lucerastat for the whole study.

4.1.7 PK trough set

The PK trough set is the subset of the SAF including all subjects who have at least one PK trough sample collected after initiation of lucerastat treatment, had evaluable plasma concentrations, and did not deviate from the protocol in a way that might affect the evaluation of the PK endpoint.

The reason for excluding a subject or a PK trough sample from analysis will be documented by the Idorsia Clinical Pharmacologist before breaking the randomization blind.

4.1.8 PK sub-study set

The PK sub-study set is the subset of the SAF including all subjects who received the morning dose of lucerastat at the Month 1 visit, have the pre-dose PK sample and pivotal post-dose PK samples collected for the 12 h PK profile, had evaluable plasma concentrations, and did not deviate from the protocol in a way that might affect the evaluation of the PK endpoints.

The reason for excluding a subject from analysis will be documented by the Idorsia Clinical Pharmacologist before breaking the randomization blind.

4.2 Usage of the analysis sets

The mFAS and the PPS will be used for the analysis of the primary endpoint.

Results based on the PPS will supplement those based on the mFAS to assess the robustness of the treatment effect. These analyses will also be produced on the FAS as a sensitivity analysis if 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 following table provides an overview of the usage of the different analysis sets.

Table 2 Overview of the different analysis sets and their usage

	FAS	mFAS	mFAS-GIS	PPS	SAF	PK trough set	PK sub-study set
Demographics, baseline and disease characteristics	✓	✓[a]	✓	✓			
Fabry disease history	✓	✓[a]					
Other medical history	✓	✓[a]					
Previous and concomitant therapies					✓		
Treatment exposure					✓		
Efficacy observation period		✓					
Primary efficacy endpoint	✓[a]	✓		✓			
Secondary efficacy endpoints related to abdominal pain and diarrhea		✓	✓				
Other secondary efficacy endpoint (Gb3)		✓					
Exploratory efficacy endpoints		✓					
Safety endpoints					✓		
Quality of life endpoints		✓					
PK trough analysis						✓	
PK profile sub-study							✓
Biomarker endpoints		✓					

[a] if FAS and mFAS differ by at least 5 subjects

FAS = Full analysis set; Gb3 = globotriaosylceramide; mFAS = Modified full analysis set; mFAS-GIS = Modified full analysis set – gastrointestinal symptoms; PK = pharmacokinetic(s); PPS = Per-protocol analysis set; SAF = Safety set.

5 GENERAL DEFINITIONS AND DERIVATIONS

5.1 Dates

'Randomization date' is taken from the date of the randomization visit (V2) recorded in the eCRF. Subjects for whom the date of the randomization visit does not match the date recorded in the IRT system will be listed.

The **'Start of treatment date / First study treatment intake date'** is defined as the date the first dose of study treatment was received (lucerastat or placebo) as documented in the eCRF study treatment log.

The **'EOT date'** is defined as the date of the last dose of study treatment intake as documented in the eCRF study treatment log. In case of incomplete date of the last dose of study treatment intake in the study treatment log of a subject having reached EOS, the EOT

date will be imputed with the latest possible date compatible with the incomplete date, while not being later than EOS.

The **'EOS date'** will be taken from the eCRF end of study status form. If this date is missing, the last recorded visit or telephone call on the eCRF is considered as the EOS date.

5.2 Baseline

Unless explicitly defined otherwise, the **'Baseline'** value for **efficacy** is defined as the last non-missing value recorded up to and including the day of the randomization visit for each endpoint and each subject individually.

5.3 Study Day

The **'Study Day'** is defined as the day relative to the day of the randomization visit, with the day of randomization visit considered as 'Day 1' of the study, the day after the randomization visit as 'Day 2' and so forth. There is no 'Day 0' in this study, so the day before the day of randomization visit is considered to be 'Day –1'.

5.4 Treatment Day

The **'Treatment Day'** is defined as the day relative to the start of treatment date, with the start of treatment date considered as 'Treatment Day 1', the day after the start of treatment date as 'Treatment Day 2' and so forth. There is no 'Treatment Day 0', so the day before the start of treatment date is considered to be 'Treatment Day –1'.

6 DEFINITION OF SUBGROUPS

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 are defined:

- Geographical region (Europe/Australia, North America).
- Age (< 65 years vs ≥ 65 years).
- Estimated glomerular filtration rate (eGFR) at screening visit (≥ 60 mL/min/1.73 m^2, < 60 mL/min/1.73 m^2).
- Urine albumin-to-creatinine ratio (UACR) at screening visit (≥ 30 mg/g, < 30 mg/g).
- Fabry disease subtype (Classic vs Late onset), as determined by FD experts.
- Sex-adjusted α-GalA activity (Male < 5%, Female < lower limit of normal [LLN] vs Male ≥ 5%, Female ≥ LLN). Subjects who did not stop ERT at least 15 days before the baseline α-GalA measurement or with the baseline measurement missing will not be assigned to either of the two subgroups.
- Mutation amenability to migalastat (Amenable vs Not amenable).

- FD complications (myocardial infarction, ischemic stroke, transient ischemic attacks, as documented in detailed information of the eCRF page: other FD-related symptoms and complications, or renal impairment defined as eGFR at screening visit < 60 mL/min/1.73 m^2) at screening (yes, no).
- Chronic pain medications (yes, no): use of the same pain medication on at least 21 days during the 4 weeks prior to randomization (study day –28 to study day –1), regardless of the number of pain medications used. Only pain medications from the following classes are considered: anti-epileptics, tricyclic antidepressants (TCAs), serotonin-norepinephrine re-uptake inhibitors (SNRIs)/atypical, selective serotonin re-uptake inhibitors (SSRIs), opioid analgesics, non-opioid analgesics.
- Chronic pain medications (monotherapy, combination, no):
  - Subjects with one pain medication taken for at least 21 days during the 4 weeks prior to the randomization visit are classified as "Monotherapy".
  - Subjects with more than one pain medication taken for at least 21 days during the 4 weeks prior to the randomization visit are classified as "Combination".
  - All other subjects are classified as "No".
  - Only pain medications from the following classes are considered: anti-epileptics, TCAs, SNRIs/atypical, SSRIs, opioid analgesics, non-opioid analgesics.
- Chronic opioids (yes, no): use of at least one opioid on at least 21 days during the 4 weeks prior to the randomization visit.
- Chronic anti-epileptics/anti-depressants (yes, no): use of at least one anti-epileptic or at least one anti-depressant on at least 21 days during the 4 weeks prior to the randomization visit.

Antidepressants are categorized as TCAs, SNRIs/atypical or SSRIs, as detailed in Table 3.

Table 3 Classes of antidepressants

Pain medication class (protocol)	eDiary category (CM.CMSCAT)	eDiary medication name
TCA	Antidepressant	Amitriptyline Nortriptyline
SNRI/atypical	Antidepressant	Duloxetine Venlafaxine Mirtazapine
SSRI	Antidepressant	Sertraline Fluoxetine Citalopram

SNRI = serotonin-norepinephrine re-uptake inhibitor; SSRI = selective serotonin re-uptake inhibitor; TCA = tricyclic antidepressant.

7 DEFINITIONS OF VARIABLES

7.1 Subject disposition

The subject disposition is detailed by the following definitions:

- Subjects screened (subjects with at least one screening date).
- Subjects re-screened (subjects with more than one screening date).
- Screen failures (subjects screened but not randomized).
- Subjects randomized (subjects with randomization number present in IRT system).
- Subjects treated (subjects with at least one dose of study treatment in eCRF study treatment log).
- Subjects who completed the study treatment (treated subjects without "Discontinuation" as reason for treatment stop in the eCRF study treatment log: record with DS.DSSCAT = END OF TREATMENT).
- Subjects who prematurely discontinued treatment (treated subjects with "Discontinuation" as reason for treatment stop in the eCRF study treatment log).
- Subjects who prematurely discontinued treatment due to the COVID-19 pandemic (treated subjects with "Discontinuation" as reason for treatment stop and "Is reason for discontinuation/interruption related to the COVID-19 pandemic?" ticked "Yes" in the eCRF study treatment log).
- Subjects who did not enter PTOP (subjects who prematurely discontinued study treatment, without any PTOP visit date).
- Subjects who entered PTOP (subjects who prematurely discontinued study treatment, with at least one PTOP visit date).
- Subjects who completed PTOP (subjects who entered PTOP, with a Month 6 PTOP visit date).
- Subjects who completed the study (subjects with "Did the subjects complete the study?" ticked "Yes" in the eCRF end of study status form: record with DS.DSSCAT = END OF STUDY).
- Subjects who completed the study without premature treatment discontinuation.
- Subjects who prematurely withdrew from the study (randomized subjects with "Did the subjects complete the study?" ticked "No" in the eCRF end of study status form).
- Subjects who prematurely withdrew from the study due to the COVID-19 pandemic (randomized subjects with "Did the subjects complete the study?" ticked "No" and "Is the reason the subject did not complete the study related to the COVID-19 pandemic?" ticked "Yes" in the eCRF end of study status form).
- Subjects who prematurely withdrew from the study prior to Month 6 (randomized subjects without Month 6 visit or Month 6 PTOP visit).

- Subjects who prematurely withdrew from the study due to the COVID-19 pandemic prior to Month 6.

7.2 Analysis sets

Analysis sets are summarized as follows:

- Subjects in the SCR
- Subjects in the FAS
- Subjects in the mFAS
- Subjects in the PPS
- Subjects in the mFAS-GIS
- Subjects in the SAF
- Subjects in the PK trough set
- Subjects in the PK sub-study set

Definitions of the analysis sets are provided in Section 4.1.

7.3 Demographics and baseline characteristics

The demographics and baseline characteristics include the following:

- Sex
- Age (years; continuous, categorical: 18–64, 65–84, ≥ 85 and over) overall and by sex
- Body weight (kg) overall and by sex
- Height (cm) overall and by sex
- Body mass index (kg/m^2) (continuous, < 18.5, 18.5 – < 25, 25 – < 30, 30 – < 35, 35 – < 40, ≥ 40)
- Childbearing potential at screening (yes, no; for female subjects)
- Reason for not being of childbearing potential (menopause, medical history; for female subjects)
- Race
- Ethnicity
- Country
- Region

The reason for not being of childbearing potential (RP.RPTESTCD = CHILDPOT and RP.ORRES = N) will be menopause for female subjects with (RP.RPTESTCD = MENOSTA and RP.ORRES = MENOPAUSE) and medical history otherwise.

7.4 Fabry disease history

The baseline disease characteristics will include the following:

- Diagnosis:
  - Time since initial FD diagnosis (years)
  - Diagnostic method (genetic test, α-galactosidase A [α-GalA] activity or both), as reported by the investigator
  - Time since first known FD symptoms (years)
  - Type of first known FD symptom
  - Incident case (yes, no, not known)
  - *GLA* gene mutation (c.), as reported by the variant scientist
  - *GLA* gene mutation (p.), as reported by the variant scientist
  - Transcript (NM_), as reported by the variant scientist
  - Mutation type, as reported by the variant scientist
  - Classification, as reported by the variant scientist
  - Inheritance, as reported by the variant scientist
  - Fabry disease subtype (Classic, Late onset, Not classified)
  - Mutation amenability to migalastat (Amenable, Not amenable, Not tested)
- Neuropathic pain symptoms (past and present):
  - Time since first neuropathic pain symptom (years)
  - Type of neuropathic pain (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 medications used to treat pain crisis in the last 12 months (anti-epileptics, TCAs, SNRIs, SSRIs, non-opioid analgesics, opioid analgesics)
- Other FD-related symptoms and complications (past and present):
  - Other neurological symptoms excluding neuropathic pain (heat intolerance, cold intolerance, hearing loss, tinnitus, vertigo, depression, other)
  - GI symptoms (diarrhea, abdominal pain / postprandial pain, abdominal discomfort, bloating, vomiting, nausea, constipation, early satiety, poor weight gain, other)
  - Eyes (corneal verticillata, corneal opacities, corneal dystrophy, lenticular opacities, retinal vein tortuosity, conjunctival vessel disorder, other)
  - Kidney (microalbuminuria, proteinuria, renal impairment, other)
  - Heart (heart valve insufficiency, arrhythmia, 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 [New York Heart Association class], other)
  - Respiratory (dyspnea at rest, dyspnea exertional, chronic cough, wheezing, chronic obstructive lung disease, other)

  - Cerebrovascular (transient ischemic attacks, ischemic stroke, white matter lesions, other)
  - Bones (osteoporosis, osteopenia, other)
  - Skin (angiokeratoma, anhydrosis, hypohydrosis, hyperhydrosis, acanthosis, hyperkeratosis, skin peeling, other)
- History of ERT infusion reaction
- eGFR slope before screening (derived from historical serum creatinine values)

Additional information from the site about detail of α-GalA activity, and GLA gene mutation (c., p.) and transcript (NM_), will be reported in the listing only.

Incident case information will be derived as yes, no, not known when APMH.MHOCCUR = Y, N, U, respectively.

Historical serum creatinine values will be used to derive corresponding past eGFR values according to the CKD-EPI formula [see protocol section 7.2.4.2.1]. Serum creatinine values in SI units (LB.LBSTRESN) will be converted to mg/dL by dividing by 88.4 without rounding and eGFR results will be rounded to the nearest integer value. For each serum creatinine value, the age at time of collection will be used to derive the corresponding eGFR. The age at time of collection will be derived from the age at screening as DM.AGE – floor [(date screening – date sample)/365.25 + 0.5], assuming that, on the average, the (unknown) birth date is 6 months before the screening date. The subject historical eGFR slope (mL/min/1.73 m^2 per year) will be estimated via a linear mixed model as described in Section 15.4.1.1 but only including time as a fixed effect, using all eGFR values up to and including the randomization visit (i.e., including values collected during the study). Subjects with eGFR values at screening and/or randomization but without any historical eGFR values will not be included in the mixed model and their historical eGFR slope will therefore be missing.

Any calculation involving a "Time since" a date of interest is defined as (Date of randomization – Date) in days / 365.25. In the event of incomplete date of interest with missing day, "Time since" is calculated as [(year of randomization – year of Date) + (month of randomization – month of Date)/12]. In the event of incomplete date of interest with missing day and month, "Time since" is calculated as (year of randomization – year of Date).

7.5 Baseline disease characteristics

The baseline disease characteristics will include the following:

- α-GalA activity (nmol/h/mg of protein) determined by central laboratory described separately by sex as a continuous variable for the normalized value (percentage of the median of normal: 100 * x / 37.8 if the LLN is 23.1; 100 * x / 19.0 if the LLN is 10.32)

and as a categorical variable (Males: < 5% of normal vs ≥ 5% of normal; Females: < LLN vs ≥ LLN).

- Plasma globotriaosylceramide (Gb3; ng/mL) and plasma lysoGb3 (ng/mL) overall and by sex.
- eGFR at screening (used to determine the starting dose of study treatment; mL/min/1.73 m^2), and categorized as ≥ 90, ≥ 60 and < 90, ≥ 45 and < 60, ≥ 30 and < 45, ≥ 15 and < 30.
- Modified BPI-SF3 "pain at its worst in the last 24 hours" score [baseline defined in Section 9.1].
- NRS-11 score of "abdominal pain at its worst in the last 24 hours" [baseline defined in Section 9.2.1].
- Number of days with at least 1 stool of BSS consistency Type 6 or 7 [baseline defined in Section 9.2.2].
- Patient Global Impression of Severity of Disease (PGIS-D) score.
- Patient Global Impression of Severity of neuropathic Pain (PGIS-P) score.

7.6 Medical history (other than FD)

Diseases and procedures except FD reported in the eCRF will be identified by selecting MH.MHCAT = GENERAL and be classified as previous or current at screening based on the question "Ongoing at screening?".

7.7 Previous and concomitant medications (other than FD-specific therapy)

Previous/concomitant medications reported in the eCRF excluding FD-specific therapy will be identified by selecting CM.CMCAT = GENERAL.

A medication will be considered as previous medication if 'started before first study treatment administration' is ticked 'Yes'.

A medication will be considered as study treatment concomitant at baseline if 'started before first study treatment administration' is ticked 'Yes' and end date (possibly missing or incomplete) is compatible with being greater than or equal to the first intake of study treatment.

A medication will be considered as study treatment concomitant if study treatment concomitant at baseline or started between first and last intake of study treatment.

A medication will be considered as study concomitant if ongoing at the day of the informed consent or initiated during the time from the day of informed consent included up to the EOS included. A medication stopped on day of informed consent will be considered to be not study concomitant.

Any pain medications recorded in the eDiary during the week up to first study treatment intake (treatment day –6 to treatment day 1) will be considered as study treatment-concomitant at baseline. Study treatment-concomitant and study-concomitant pain medications are defined as described above for the medications recorded in the eCRF.

7.8 Previous FD-specific therapy

Previous FD therapy will be identified by selecting CM.CMCAT = PREVIOUS FABRY DISEASE SPECIFIC THERAPY.

7.9 ERT infusion during study

Initiation or re-initiation of ERT during the study will be identified by selecting CM.CMCAT = ERT INFUSION DURING THE STUDY.

8 TREATMENT EXPOSURE / DURATION OF EFFICACY OBSERVATION PERIOD

8.1 Duration of exposure and mean daily dose

Duration of exposure to study treatment (months) is defined as (Date of last study treatment intake – Date of first study treatment intake + 1 [in days]) / (365.25/12), regardless of any treatment interruptions. If the first study treatment intake is on the same day as the randomization date, the duration of exposure is reduced by 0.5 day. If the last study treatment intake is on the same day as the Month 6 visit, the duration of exposure is reduced by 0.5 day.

Treatment dates will be taken from the eCRF study treatment log.

The study treatment is administered b.i.d., i.e., the daily dose of a given day is twice the dose (mg) recorded in EX.EXDOSE if this is a day without dose change. A day with dose change is identified when being simultaneously the last day of a constant-dosing interval of EX and the first day of the next constant-dosing interval; in that situation, the daily dose is the sum of the two distinct doses. On days without dose change, if the dose frequency is reported as QD or ONCE, only one dose is counted on the respective day.

Only one dose (the evening dose) is counted on Treatment Day 1 (unless the first study treatment intake is not on the same day as the randomization date; in this situation two doses are counted on Treatment Day 1). Only one dose is counted on the EOT date (unless the last study treatment intake is not on the same day as the Month 6 visit; in this situation two doses are counted on the EOT date).

8.2 Study treatment discontinuation

A premature permanent study treatment discontinuation is defined as presence of "Discontinuation" as reason for treatment stop in the eCRF study treatment log.

Reasons for premature treatment discontinuation are as follows:

- Adverse event (AE)
- Pre-specified study treatment discontinuation criteria
- Lack of efficacy
- Withdrawal by subject
- Lost to FU
- Death
- Other

In addition, the site has to indicate if the reason for study treatment discontinuation is related to the COVID-19 pandemic.

8.3 Study treatment compliance

As outlined in the protocol section 5.1.7.2, the study treatment compliance will be defined for each interval between two visits (hereafter labeled as "period") as (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 (count of capsules = SUPPDM.QVAL where SUPPDM.QNAM = DOSERGM).

In the event of study treatment adjustment, 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 subject was contacted for dose adjustment, as documented in the study treatment log (EX.EXSTDTC where EX.EXADJ = ADJUSTED BY INVESTIGATOR in the EX record of the new constant-dosing interval).

The period considered for the interval (Visit n, Visit n+1) is the time from Visit n included to Visit n+1 excluded (remote visits performed instead of site visits due to COVID-19 are also considered for the derivation of compliance). As a consequence, the number of days in the period is defined as the difference of dates between Visit n+1 (or last study treatment intake if EOT visit) and Visit n (or first study treatment intake if randomization visit). The number of times the study treatment should have been taken at the prescribed dose regimen in the period is calculated as the number of days in the period × 2 with the correction that study treatment is taken only in the evening of the randomization visit [protocol section 5.1.2]. In situations of two successive constant-dosing intervals where the end date of the first interval is also the start date of the second interval, the dose of the first interval will be taken as the prescribed dose of the morning of that common day and the dose of the new interval will be taken as the prescribed dose of the evening of that common day.

In addition, overall compliance from the day of the randomization visit (included) to the day of the EOT visit (excluded) will be reported.

In order to compensate for potential completion issues in the eDiary data, the study treatment compliance according to study treatment log will also be derived the same way.

8.4 Duration of efficacy observation period

Duration of efficacy observation period (months) is defined as (date of last visit performed up to Month 6 [including unscheduled visits and EOT visit if applicable; if no such visit is available, the EOT date will be used] – Randomization date + 1 [in days]) / (365.25/12).

Duration of PTOP (months) is defined as (last PTOP visit date – [EOT date + 1 day] + 1 [in days]) / (365.25/12).

8.5 Study withdrawal

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.

Study withdrawals are subjects having not completed the study (defined as subjects with "Did the subjects complete the study?" ticked "No" in the eCRF end of study status form).

Possible reasons for study withdrawal are as follows:

- Withdrawal by subject
- AE
- Lost to FU
- Death
- Other

In addition, the site has to indicate if the reason for study withdrawal is related to the COVID-19 pandemic.

9 EFFICACY VARIABLES

9.1 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".

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 (used in sensitivity and supportive analyses) will be derived with the same validity conditions.

The selection of daily scores over 4 weeks for each time point is described in Table 4.

Table 4 Daily scores used for derivation of monthly scores

Monthly score	First study day	Last study day
Baseline	–28	–1
Month 1	3	30
Month 2	34	61
Month 3	64	91
Month 4	95	122
Month 5	125	152
Month 6	Month 6 visit* – 28 days	Month 6 visit* – 1 day

* If Month 6 visit is on Day 198 or later (i.e., out of protocol defined visit window), use Day 169 to 196 instead. If Month 6 visit is on Day 168 or earlier (i.e., out of protocol defined visit window), the Month 6 score will be missing.

It may be noted that for subjects with a Month 6 visit performed earlier than Day 181, there will be an overlap between the 28 days used to derive the Month 5 monthly score and the 28 days used to derive the Month 6 monthly score.

9.2 Secondary efficacy endpoints

As outlined in protocol section 10.2.2, 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.

9.2.1 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

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 9.1].

The variable will be derived for all subjects and displayed in data listings but used only on mFAS-GIS.

9.2.2 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

Using the number of 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" (i.e., "number of days with diarrhea") 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 for all subjects and displayed in data listings but used only on mFAS-GIS. 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 in the same way.

9.2.3 Change from baseline to Month 6 in plasma Gb3 (ng/mL)

Plasma Gb3 data will not be available for statistical analysis until the study database is locked and the randomization code is broken.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

Table 5 Time windows for visit re-mapping

Visit	Study day (nominal value)	Lower limit study day	Upper limit study day
Baseline	1	Day of screening visit	1
Month 1	30	2	45
Month 2	61	46	75
Month 3	91	76	106
Month 4	122	107	136
Month 5	152	137	167
Month 6	183	168	213

Should more than one value fall within the same time window, then the closest value to the planned study day will be assigned to the visit. If the values are equidistant to the planned study day, the latest value will be retained.

Using the plasma Gb3 data collected at baseline and Month 6, the change from baseline to Month 6 will be calculated as the difference (Month 6 – baseline) and also as the percent change from baseline (100 × [Month 6 – baseline] / baseline).

Values reported as below the limit of quantification (BLQ) will be set to the limit of quantification in the analyses. The number of values reported as BLQ by treatment group and visit will be included in the tables.

9.3 Renal function endpoints

9.3.1 Subject eGFR slope from baseline to Month 6

Using all eGFR values from baseline to Month 6, the subject eGFR slope (mL/min/1.73 m^2 per year) will be estimated via a linear mixed model, as described in Section 15.4.1.1.

9.3.2 Change from baseline to Month 6 in UACR

UACR will be derived by dividing urine albumin by urine creatinine. Urine albumin values reported as BLQ will be set to the limit of quantification and values reported as > x.x (above x.x) will be set to x.x (equal to x.x) before deriving UACR.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.4 Echocardiography-based endpoints

The echocardiography variables are listed in Table 6.

Table 6 Echocardiography variables

Variable	Test name in SDTM data
LVM indexed to Height ($g/m^{2.7}$)	Left Ventricular Mass Height2.7 (LVMH)
LVM indexed to BSA (g/m^2)	Left Ventricular Mass Index (LVMASIDX)*
Left Ventricular Posterior Wall Thickness (mm)	Posterior Wall Thickness - End Diastole (LVPWD)
Left Ventricular Interventricular Septum Thickness (mm)	Cross-sec Thickness, EVD (THCKEVD)
Left Ventricular Mean Wall Thickness (mm)	Mean Wall Thickness (LVMWT2D)
LVEF (%)	Left Ventricular Ejection Fraction (LVEF)
Left Ventricular End Diastolic Volume indexed to BSA (mL/m^2)	Left Ventricle End Diastolic Volume BP (EDVB)*
Left Ventricular End Systolic Volume indexed to BSA (mL/m^2)	Left Ventricle End Systolic Volume BP (ESVB)*
Left Atrial Volume indexed to BSA (mL/m^2)	Left Atrium - End Systolic Volume BP (LAB)*

* Variables provided by vendor are not indexed to BSA and will therefore be indexed to BSA as described below.

BSA will be derived using the Mosteller formula:

$$BSA(m^2) = \sqrt{\frac{height\ (cm) * weight\ (kg)}{3600}}$$

Height at screening will be used. The last available weight measurement collected up to or on the date of the respective echocardiography assessment will be used. Variables will be indexed to BSA by dividing the measurement by BSA, if applicable [see Table 6].

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5, with the exception that the upper limit study day for baseline will be Day 14, i.e., re-tests occurring within 14 days (included) after the randomization visit, will also be considered as baseline value for the echocardiography endpoints, if no value on or prior to Day 1 is available. Re-tests are performed if the original assessment from randomization is not evaluable or declined by the independent central reader as being of poor quality. Using these re-test assessments as baseline value is acceptable because no changes in echocardiography endpoints are expected in such a short period of 14 days.

For each echocardiography-based endpoint, absolute values and changes from baseline to Month 6 (expressed as difference and expressed as percent change) will be summarized by treatment group and visit.

The change from baseline to Month 6 of each parameter will be calculated as the difference (Month 6 – baseline). In addition, the percent change from baseline to Month 6 will be calculated as 100 × (Month 6 – baseline) / baseline.

9.5 Pain medication endpoints based on daily entries in eDiary

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

Opioids are selected using a list of WHO Drug Dictionary codes maintained in a separate file which will be finalized and stored in the eTMF before breaking the randomization blind.

First, the dose of all opioids will be transformed to the same unit (mg), using Table 7, as needed. Doses will then be converted to equianalgesic doses of oral morphine. The above-mentioned file which includes the list of opioids also includes the corresponding conversion factors.

Table 7 Conversion of units to mg

Original unit	Converted unit
FINGERTIP UNIT	400 mg
tsp (teaspoon)	5 mL
tbsp (tablespoon)	15 mL
DROP	0.05 mL

mL is converted to mg by multiplying the volume by the concentration.

For each day on which a subject reported at least one opioid in the eDiary, the total daily dose of opioid analgesics will be derived as the sum of all converted doses for the respective day. For days on which a subject did not report any opioids, the total daily dose of opioid analgesics will be imputed as 0 mg (i.e., no opioids taken on that day) if the subject did record at least one *non-opioid* pain medication in the eDiary on the respective day.

If a subject did not report any pain medications in the eDiary on the respective day, the total daily dose of opioid analgesics will be imputed as described in Table 8, based on the eDiary question "Did you take any pain medication in the last 24 hours?". If a subject replied to this question more than once on a given day, the following algorithm will be applied to retain only one answer per day:

- If there is at least one "Yes", disregard all "No";
- In a second step, only keep the last answer of the day.

Table 8 Imputation of total daily dose of opioid analgesics based on answer to "Did you take any pain medication in the last 24 hours?" if no pain medications have been recorded in the eDiary

Answer on Day X at time YY:YY	Conclusion	Imputed total daily dose of opioid analgesics
"Yes" + at least 1 medication recorded on Day X–1 after time YY:YY	No pain medication was taken on Day X	0 mg
"No"		
"Yes" + no medication recorded on Day X–1 after time YY:YY	Unknown if any pain medication was taken on Day X	Missing
Not available / missing		

The mean weekly dose at baseline is then derived as 7 * (sum of all total daily doses from study day –28 to –1) / number of days with non-missing total daily dose. Post-baseline, the mean weekly dose is derived in the same way, for each month, using the study days as defined in Table 4. In addition, the overall mean weekly dose from baseline to Month 6 is derived as the mean of the mean weekly dose at Month 1, Month 2, Month 3, Month 4,

Month 5 and Month 6. This overall score will only be derived for subjects who have a non-missing score available at each month.

By only using days with a non-missing total daily dose of opioid analgesics to derive the mean weekly dose, the implicit assumption is made that the total daily dose of opioid analgesics on days with missing data is the same as the average total daily dose of opioid analgesics on days with data recorded.

9.5.2 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 anticonvulsants (anti-epileptics), antidepressants (TCAs, SNRIs, SSRIs), or opioid analgesic drugs.

This endpoint is a binary variable (yes/no) where yes corresponds to at least 1 day on significant rescue pain therapy as reported in the eDiary by the subject between randomization and the Month 6 visit inclusive.

An initiation or dose escalation is defined as described in Table 9. A day with at least one initiation or dose escalation is a day with use of significant rescue pain therapy.

Table 9 Significant rescue pain therapy: definition of initiation and dose escalation

Initiation	Opioid analgesics	Only defined for subjects who have not taken any opioid analgesics between study day –28 and –1 as any day from study day 1 onwards with a total daily dose of opioid analgesics > 0 mg.
	Anticonvulsant / Antidepressant	For each anticonvulsant / antidepressant, any day from study day 1 onwards with an anticonvulsant / antidepressant medication recorded which has not been taken between study day –28 and –1.
Dose escalation	Opioid analgesics	Any day from study day 1 onwards with a total daily dose of opioid analgesics > maximum total daily dose of opioid analgesics between study day –28 and –1.
	Anticonvulsant / Antidepressant	For each anticonvulsant / antidepressant, any day from study day 1 onwards with an anticonvulsant / antidepressant medication with a daily dose > maximum daily dose between study day –28 and –1.

Days with missing diary entries (derived using Table 8), are only considered as days with significant rescue pain medication if the closest available non-missing day before the day in question is a day with significant rescue pain medication (derived using Table 9) and the subject answered “Too much neuropathic pain” to the question “You missed at least one diary. Why?” on the closest available non-missing day after the day in question.

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

The annualized rate of days with significant rescue therapy (ARRT) is defined as the number of days on significant rescue pain therapy as reported in the eDiary per subject-year from randomization up to the Month 6 visit inclusive.

For the statistical analysis of the ARRT, the following two variables will be used:

- The subject's number of days on significant rescue pain therapy as reported in the eDiary up to the Month 6 visit inclusive;
- The observation time expressed in years as the total number of days with non-missing eDiary entries up the Month 6 visit inclusive, divided by 365.25.

Days with significant rescue pain therapy are derived as described in Section 9.5.2.

9.6 Clinical symptom endpoints based on data collected at site visits

9.6.1 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")

Using the data collected at baseline and Month 6, the change from baseline to Month 6 will be calculated as the difference (Month 6 – baseline). In addition, the percent change from baseline to Month 6 will be calculated as 100 × (Month 6 – baseline) / baseline.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.2 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")

Using the data collected at baseline and Month 6, the total score at baseline and the total score at Month 6 will be derived as the sum of the 7 questions.

If one question or more is unanswered the total score will be missing. The change from baseline to Month 6 will be calculated as the difference (total score at Month 6 – total score at baseline).

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.3 Change from baseline to Month 6 in the subject's rating of severity of neuropathic pain as measured by the PGIS-P

The change in severity of neuropathic pain from baseline to Month 6 will be calculated as the PGIS-P value observed at Month 6 minus the baseline value.

Subjects with a negative change from baseline (change in PGIS-P < 0) will be considered as "improvement", while a change ≥ 0 will be considered as "no improvement".

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.4 Subject rating of change in neuropathic pain severity since study treatment start as measured by the PGIC-PS at Month 6

Three response variables (yes/no), each corresponding to a different level of improvement, will be defined using the observed data at Month 6:

- "minimally improved" or better (Response = Yes for PGIC-PS = 1, 2 or 3; Response = No for PGIC-PS > 3);
- "much improved" or better (Response = Yes for PGIC-PS = 1 or 2; Response = No for PGIC-PS > 2);
- "very much improved" (Response = Yes for PGIC-PS = 1; Response = No for PGIC-PS > 1).

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.5 Change from baseline to Month 6 in the subject's rating of disease severity as measured by the PGIS-D

The change in severity of disease from baseline to Month 6 will be calculated as the PGIS-D value observed at Month 6 minus the baseline value.

Subjects with a negative change from baseline (change in PGIS-D < 0) will be considered as "improvement", while a change ≥ 0 will be considered as "no improvement".

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.6 Subject rating of change in disease severity since study treatment start as measured by the PGIC-DS at Month 6

Three response variables (yes/no), each corresponding to a different level of improvement, will be defined using the observed data at Month 6 using the approach described for PGIC-PS in Section 9.6.4.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

9.6.7 Change from baseline to Month 6 in the total score of the subject's rating of the CESD-R-20

Using the data collected at baseline and Month 6, the total score at baseline and the total score at Month 6 will be derived as the sum of the 20 questions. In order to make the revised

CESD-R have the same range as the original version (i.e., the 'CESD style score'), the values for the top two responses are given the same value (i.e., answers with the value 4 are given the value 3). As in the original CESD, the range of possible scores is between 0 and 60.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

If one question or more is unanswered the total score will be missing. The change from baseline to Month 6 will be calculated as the difference (total score at Month 6 – total score at baseline).

Since a total score ≥ 16 indicates a person at risk of clinical depression, a variable for change in risk of depression between baseline and Month 6 will be defined as decreased risk (at risk at baseline, not at risk at Month 6), no change (at risk at baseline and Month 6 or not at risk at baseline and Month 6), increased risk (not at risk at baseline, at risk at Month 6).

In addition to the binary classification of the total score (<16, ≥16), the 5-level classification will also be derived [Radloff 1977]:

- Meets criteria for major depressive episode: anhedonia or dysphoria nearly every day for the past two weeks (defined as at least 1 question out of 2, 4, 6, 8 and 10 answered "nearly every day for 2 weeks"), plus symptoms in an additional 4 DSM symptom groups noted as occurring nearly every day for the past two weeks (defined as at least 1 question answered "nearly every day for 2 weeks" in at least 4 additional symptom groups);
- Probable major depressive episode: anhedonia or dysphoria nearly every day for the past two weeks (defined as above), plus symptoms in an additional 3 DSM symptom groups reported as occurring either nearly every day for the past two weeks, or 5–7 days in the past week (defined as at least 1 question answered "5–7 days" or "nearly every day for 2 weeks" in at least 3 additional symptom groups) without meeting the criteria for major depressive episode;
- Possible major depressive episode: anhedonia or dysphoria nearly every day for the past two weeks (defined as above), plus symptoms in an additional 2 DSM symptom groups reported as occurring either nearly every day for the past two weeks, or 5–7 days in the past week (defined as at least 1 question answered "5–7 days" or "nearly every day for 2 weeks" in 2 additional symptom groups);
- Subthreshold depression symptoms: total CESD-style score of at least 16 without meeting the above criteria;
- No clinical significance: total CESD-style score of less than 16 across all 20 questions without meeting above criteria.

9.7 Treatment failure from baseline up to Month 6

The time to treatment failure up to Month 6 will be defined as the time between randomization and the earliest of the following:

- The date of ERT initiation or re-initiation;
- The date of permanent study treatment discontinuation for any reason.

Subjects who initiated ERT or permanently discontinued study treatment for any reason will be considered as "treatment failure" (event) while other subjects will be censored at their date of last visit performed up to Month 6.

The time to treatment failure will be expressed in months and derived as [Date – Date of randomization] in days / (365.25/12).

10 SAFETY VARIABLES

For determination of treatment-emergent events, the safety analysis will retain the AEs with an onset date between the start of treatment date (included, provided that AE onset is not prior to first dose of study treatment, i.e., AE.AESTRTPT is different from BEFORE) and EOT + 30 days (included). AEs with missing or incomplete onset date compatible with being between the start of treatment date (included, provided that AE onset is not prior to first dose of study treatment, i.e., AE.AESTRTPT is different from BEFORE) and EOT + 30 days (included) will be considered treatment emergent.

For determination of treatment-emergent events, the safety analysis will retain the laboratory values, vital signs, and ECGs with a sampling or assessment date between the start of treatment date (excluded) and EOT + 30 days (included). The baseline value will be taken as the last value available up to the start of treatment date (included).

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

10.1 Adverse events

- Treatment-emergent AEs;
- Treatment-emergent serious AEs (SAEs);
- AEs leading to permanent discontinuation of study treatment.

10.2 Laboratory data

- Change from baseline to each visit up to Month 6;
- Treatment-emergent marked abnormalities;

Laboratory analyses are based on data received from the central laboratory as well as local laboratories. The laboratory parameters considered as safety parameters are the following:

Hematology

- Hemoglobin (g/L);
- Hematocrit (%);
- Erythrocyte count (10^{12}/L);
- Reticulocyte count (10^9/L);
- Leukocyte count (10^9/L);
- Neutrophils (10^9/L), lymphocytes (10^9/L), monocytes (10^9/L), eosinophils (10^9/L), basophils (10^9/L);
- Platelet count (10^9/L).

Blood chemistry

- Alanine aminotransferase (U/L), aspartate aminotransferase (U/L), alkaline phosphatase (U/L);
- Bilirubin (µmol/L);
- Direct bilirubin (µmol/L);
- Creatinine (µmol/L);
- eGFR (mL/min/1.73 m^2);
- Urea nitrogen (mmol/L);
- Urate (µmol/L; labelled "Uric acid" in source data);
- Albumin (g/L), Protein (g/L);
- Glucose (mmol/L), HbA1c (%);
- Cholesterol (mmol/L), triglycerides (mmol/L);
- Sodium, potassium, chloride, calcium (mmol/L);
- N-terminal pro-brain natriuretic peptide (pg/mL; pg/mL is same as ng/L).

Cardiac enzymes

- High sensitivity troponin T (ng/L).

Urinalysis

- Urine Albumin (mg/L);
- Urine Creatinine (mmol/L);
- UACR (mg/g; mg/g is same as g/kg);
- Results of the dipstick analysis.

Male reproductive hormones (local protocol)

- Total testosterone (nmol/L);
- Free testosterone (pmol/L);
- Follicle-stimulating hormone (IU/L);
- Luteinizing hormone (IU/L);
- Inhibin B (ng/L).

Semen analysis

- Sperm concentration (10^6/mL);
- Ejaculate volume (mL);
- Total sperm number (10^6/ejaculate): derived as Sperm concentration (10^6/mL) × Ejaculate volume (mL);
- Sperm motility (%);
- Normal sperm / total sperm (%).

Data in conventional units for hematocrit, N-terminal pro-brain natriuretic peptide (NT-proBNP), and UACR [respectively %, ng/L, and g/kg] will be derived from SUPPLB dataset merged by USUBJID and LBSEQ with LB dataset (using SUPPLB.IDVARVAL to match LB.LBSEQ and QNAM; LBCVRES, QNAM.LBCVRESU, QNAM.LBCVNRLO, and QNAMLBCVNRHI to get value, unit, and normal range).

10.3 Vital signs

- Change from baseline to each visit up to Month 6.
- Treatment-emergent marked abnormalities.

Vital signs include systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), mean arterial pressure (MAP; mmHg), heart rate (bpm) and body weight (kg). MAP will be derived as (SBP + 2 × DBP) / 3.

10.4 12-lead ECGs

- Change from baseline to each visit up to Month 6
- Change from pre-dose to 2 hours and 4 hours post-dose at Month 1
- Treatment-emergent marked abnormalities.

12-lead ECG parameters include heart rate (HR; bpm), PR (ms), QRS (ms), QT (ms), QTc according to Bazett's formula (QTcB; ms), QTc according to Fridericia's formula (QTcF; ms), and any morphological abnormalities as defined by the central ECG vendor.

Quantitative results (HR, PR, QRS, QT, QTcB, QTcF) will not be available for hard copy ECGs read by the central ECG vendor (only the interpretation will be available).

11 QUALITY OF LIFE ENDPOINTS

Using the SF-36v2 data collected at baseline and Month 6, the z-score (with reference to a US adult population [Ware 2000]) of each of the 8 domains (physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, mental health) and the 2 component scores (physical health, mental health) are derived at baseline and Month 6 from the 36 source items. Finally, the 8 domain z-scores and the 2 component scores are transformed to T-scores via the norm-based (50, 10) scoring [derivations are detailed in Appendix A].

The change from baseline to Month 6 of a score will be calculated as the difference (T-score at Month 6 – T-score at baseline). In the event of a missing Month 6 score (after application of the SF-36 scoring rules), the score will be left missing in the analysis.

The health transition item (much better than one year ago, somewhat better than one year ago, about the same as one year ago, somewhat worse than one year ago, much worse than one year ago) will be used without transformation. In the event of a missing Month 6 health transition item, the item will be left missing in the analysis.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

12 PHARMACOKINETIC ENDPOINTS

Lucerastat plasma concentrations will not be available for analysis of PK parameters until the study database is locked.

For PK analyses, values BLQ will be imputed with 0.

If a time of PK sampling is missing, no imputation will be applied.

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

The trough plasma concentration of lucerastat will be assessed at each visit up to Month 6 (i.e., at Months 1, 3, 5, and 6).

12.2 PK samples collected at Month 1 (PK profile sub-study)

A 12-hour PK profile will be obtained from subjects participating in the PK sub-study at the Month 1 visit. The following endpoints are defined:

- The area under the plasma concentration-time curve during one dosing interval (AUC_{τ}),
- The maximum plasma concentration (C_{max}) during one dosing interval,
- The time to reach maximum plasma concentration (t_{max}) during one dosing interval,
- The apparent terminal elimination half-life ($t_{½}$).

They will be derived from the concentration-time data by the Idorsia Clinical Pharmacologist.

13 BIOMARKERS OF FABRY DISEASE AND LUCERASTAT MECHANISM OF ACTION

As for plasma Gb3, these data will not be available for statistical analysis until the study database is locked and the randomization code is broken.

As for plasma Gb3, the data collected at baseline and Month 6 for plasma lysoGb3 (ng/mL), GlcCer (ng/mL), LacCer (ng/mL) and urine Gb3 and lysoGb3 (both normalized to creatinine) will be used to derive their change from baseline to Month 6 as the difference (Month 6 – baseline) and also as the percent change from baseline (100 × [Month 6 – baseline] / baseline).

Urine Gb3 and urine lysoGb3 will be normalized as follows:

- Normalized urine Gb3 (µmol Gb3/mol creatinine) = (10^3/1024.3) × Gb3 (ng/mL) / urine creatinine (mmol/L)
- Normalized urine lysoGb3 (nmol lysoGb3/mol creatinine) = (10^6/785.91) × lysoGb3 (ng/mL) / urine creatinine (mmol/L)

Values reported as BLQ will be set to the limit of quantification in the analyses. The number of values reported as BLQ by treatment group and visit will be included in the tables.

The values collected during the study will be mapped to protocol visits according to the windowed times described in Table 5.

14 STATISTICAL ANALYSES

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

Data will be listed and summarized using appropriate descriptive statistics:

- Number of non-missing observations, mean, standard deviation (SD), minimum, Q1, median, Q3 and maximum for continuous variables.
- Number of events, number of censored observations and Kaplan-Meier estimates of the survival function for time-to-event variables.
- Number of non-missing observations and frequency with percentage per category for categorical variables. Denominators for percentages are the number of non-missing subjects in the pertinent analysis set and treatment group, unless otherwise specified.

The number of missing values will be displayed only if > 0 and only for categorical variables, after the last category.

Absolute change from baseline is defined as: post-baseline value minus baseline value, i.e., a positive sign indicates an increase compared to baseline.

A percentage change from baseline is defined as the absolute change from baseline divided by the baseline value (if the baseline value is > 0) and then multiplied by 100. In the event of baseline and post-baseline value both equal to 0, the ratio to baseline will be set to 1 (i.e., the percent change from baseline will be set to 0%). If baseline is equal to 0 and post-baseline value greater than 0 (infinite ratio) or if baseline greater than 0 and post-baseline equal to 0 (log[ratio] = minus infinity), the percent change will be missing.

Summary statistics of percentage changes will be derived from the geometric mean of the ratio to baseline transformed into a percentage change by using the transformation $x \rightarrow (x-1) \times 100$. The CV of the geometric mean will be derived as described in Appendix B.

In tables, column labels will be 'Lucerastat' and 'Placebo' and a total column is added in baseline disease characteristics and demography.

SAS/STAT® version 14.1 or higher will be used for all statistical analysis [SAS Institute 2015].

14.1 Disposition

The number of 'Subjects Screened' will be summarized by country and site along with the number and percentages of 'Subjects Re-Screened', 'Screening Failures', and 'Randomized Subjects' with percentages based on the SCR.

The reasons for screen failures will be summarized on the SCR showing the number of subjects screened, the number of screen failures, the number of subjects re-screened, the number of re-screen failures and the primary reason for screen failure (the last screen failure in case of re-screen). A listing will display the reason for (the last) screen failure and the date of (the last) screen failure. A separate listing will display the inclusion/exclusion criteria not met of screen failures for which the reason is "not eligible per inclusion/exclusion criteria".

The disposition of the randomized subjects will be summarized on the FAS by study treatment status and study completion status as detailed in Section 7.1 (without counts of screened subjects, re-screened subjects and screen failures). In the event of randomized subjects having not started study treatment, the summary will provide the list in a footnote.

The disposition of treated subjects will be summarized similarly on the SAF (without counts of screened subjects and randomized subjects). In the event of randomized subjects taking the incorrect treatment, the summary will provide the list of subjects that have switched treatment groups compared with the mFAS.

Subjects unblinded during study (if any) will be detailed (event that triggered the request, action taken following the unblinding) in a listing.

Subjects discontinuing treatment permanently will be summarized on the mFAS and the SAF, by treatment and overall along with reasons for discontinuing study treatment. This summary will be repeated for study treatment discontinuations related to the COVID-19 pandemic.

Subjects withdrawing from the study early will be summarized on the FAS, by treatment and overall along with the reasons for withdrawal from the study. This summary will be repeated for study discontinuations related to the COVID-19 pandemic.

Eligibility criteria not met will be summarized on the FAS by treatment and overall. Eligibility criteria not met will also be summarized on the subset of the SCR of screen failures not eligible as per inclusion/exclusion criteria.

Protocol deviations will be summarized on the FAS, by treatment and overall, by protocol by category, sub-category (ordered according to the first 3 digits of the deviation identifier). Important protocol deviations (DV.DVGRPID = IMPORTANT) will be summarized the same way.

The summary of protocol deviations and important protocol deviations will be repeated for deviations related to the COVID-19 pandemic.

The number and percentage of 'Subjects included' in each analysis set will also be summarized with percentages based on the relevant set: (FAS for mFAS, mFAS-GIS, and PPS; SAF for PK through set and PK sub-study set). A separate table will provide, for each analysis set, the number and percentage of subjects excluded by reason for exclusion.

Subjects with a start of treatment date not matching the randomization date of the IRT system will be listed.

14.2 Demographics and baseline characteristics

Demographics and baseline characteristics will be summarized on the FAS, mFAS (if it differs by at least 5 subjects), PPS, and mFAS-GIS populations, by treatment and overall, first based on all subjects and then by sex.

The country will be displayed using the ISO 3166 short name lower case, omitting "(the)" if present in the short name.

14.3 Analysis of stratification variable

In the event of discrepancies between the IRT system and the eCRF, each of the two stratification variables will be summarized (on the mFAS, PPS, and mFAS-GIS populations, by treatment and overall) in categories for both the IRT-assigned value and the value reported in the eCRF. In addition, shift tables of ERT status at screening according to eCRF (table rows) by ERT status at screening according to IRT (table columns) will be prepared for each treatment group and overall, first based on all subjects

and then separately for each sex (according to eCRF). Otherwise, the stratification variables will be summarized only once.

The stratum information recorded in the IRT system will be decoded from the leading digit of the randomization number (DS.DSREFID) as follows:

- 1: Sex = M, ERT at screening = "switch"
- 2: Sex = M, ERT at screening = "pseudo-naïve" / "treatment naïve"
- 3: Sex = F, ERT at screening = "switch"
- 4: Sex = F, ERT at screening = "pseudo-naïve" / "treatment naïve".

The ERT status at screening according to the eCRF will be determined by checking the medication in the CM dataset where CM.CMCAT = "PREVIOUS FABRY DISEASE SPECIFIC THERAPY" and (CMCLAS="ENZYMES" or CMDECOD in ["PEGUNIGALSIDASE ALFA","MOSS-AGAL"]) and the corresponding end date (CM.CMENDTC [possibly incomplete] vs the screening date [last screening in the event of re-screening]). In the event of missing end date, ERT status will be presumed to be "switch".

14.4 Baseline disease characteristics

Baseline disease characteristics will be summarized on the FAS, mFAS (if it differs by at least 5 subjects) PPS, and mFAS-GIS populations, by treatment and overall.

14.5 Exposure

The duration of exposure to study treatment will be presented as a continuous variable on the SAF, by treatment and overall. In addition, the cumulative distribution by different class intervals (i.e., at least 1 month, at least 2 months, at least 3 months, and so on up to 6 months, defining a month as 365.25/12 days) will be tabulated to show counts and percentages of subjects in each class interval.

The sum of duration of exposure across all subjects in years will be displayed as subject year exposure.

The mean daily dose (mg) will also be presented as a continuous variable.

14.6 Efficacy observation period and PTOP

The duration of the efficacy observation period and of the PTOP will be presented as a continuous variable on the mFAS, by treatment and overall.

14.7 Drug accountability

Study treatment dispensing and accountability data collected in the eCRF will be presented in subject listings without any further summarization.

14.8 Study treatment compliance

The eDiary-based study treatment compliance will be summarized overall and by period (V2 to V3, V3 to V4, V4 to V5, V5 to V6, V6 to V7, and V7 to V8).

The study treatment compliance according to the study treatment log will be summarized in the same way.

14.9 FD history

The FD history will be summarized on the FAS, mFAS (if it differs by at least 5 subjects) populations, by treatment and overall, first based on all subjects and then by sex.

Neuropathic pain symptoms with pre-specified answer "Other" ticked (record in SUPPMH with QNAM=PAINOTH) will be reported in summary tables on common row "Other", further details (free text in SUPPMH.QVAL) being reported in a listing. Neuropathic pain symptoms(s) triggering factors with pre-specified answer "Other" ticked (1 record in FAMH with FAORRES with FAOBJ = Other neuropathic symptoms) will be reported in summary tables on a single common row "Other", further details (free text in FAMH. FAORRES) being reported in a listing.

Other FD-related symptoms and complications will be summarized first including all symptoms and complications (previous and current) and then restricting the summary to current medical symptoms and complications. A medical symptom/complication will be retained as current if not being answered "No" to question "Ongoing at Screening" (i.e., MH.MHENRTPT = ONGOING or missing). Symptoms within a pre-specified category with the pre-specified answer "Other" ticked (MH.MHGRPID = OTHER) will be reported in summary tables on a single common row labelled "OTHER" with a breakdown by preferred term within their respective pre-specified category, further details (verbatim term: MH.MHTERM) being reported in a listing. Symptoms entered in the pre-specified category "OTHER" (MH.MHSCAT = OTHER) will be reported in summary tables within the category "OTHER" with the free text entered by the investigator (MH.MHTERM) reported in a listing.

The history of ERT infusion reaction will be summarized by system organ class (MH.MHBODSYS) and preferred term (MH.MHDECOD) in two separate tables: one for subjects with switch ERT status at screening and one for subjects with (pseudo-)naïve ERT status at screening.

The historical eGFR slope will be summarized as a quantitative variable.

14.10 Medical history (other than FD)

Diseases and procedures will be summarized on the FAS, mFAS (if it differs by at least 5 subjects) populations, by treatment and overall, by system organ class (SOC) and preferred term, first including all medical conditions (previous and current) and then restricting the summary to current medical conditions. A medical condition will be retained

as current if not being answered "No" to question "Ongoing at Screening" (i.e., MH.MHENRTPT = ONGOING or missing).

14.11 Previous and concomitant medications

Study treatment concomitant medications (other than FD-specific therapy) at baseline, study treatment concomitant medications, and study concomitant medications will be summarized on the SAF, by treatment and overall, by Anatomical Therapeutic Chemical class and preferred name.

Study treatment concomitant pain medications at baseline, study treatment concomitant pain medications, and study concomitant pain medications recorded in the eDiary will be summarized on the SAF, by treatment and overall, by Anatomical Therapeutic Chemical class and preferred name.

Previous FD-specific therapies will be summarized as well.

Initiation (or re-initiation) of ERT during study will be summarized by ERT status at baseline and overall.

15 EFFICACY ANALYSIS

15.1 Overall testing strategy

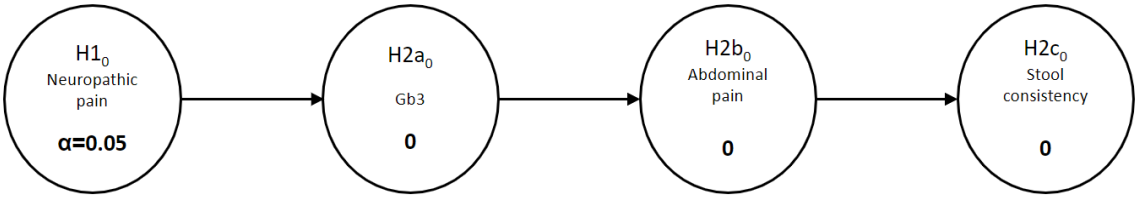
Comparisons of lucerastat vs placebo will be conducted for the primary and secondary endpoints assessed at Month 6.

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 1 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 $H1_0$ is initially assigned the local two-sided statistical significance level alpha = 0.05, whereas $H2a_0$ through $H2c_0$ from the secondary family of endpoints are assigned the local statistical significance level 0. If $H1_0$ is rejected, the local statistical significance level α is passed on to $H2a_0$ and so on as long as hypotheses are rejected.

15.2 Analysis of the primary efficacy variable

15.2.1 Hypotheses

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

$H1_0$: lucerastat – placebo = 0

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

$H1_A$: lucerastat – placebo ≠ 0

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

15.2.2 Description of missing data

All available eDiary data collected from baseline (last four weeks prior to randomization), and after randomization and up to the Month 6 visit (i.e., during the treatment phase, after treatment discontinuation and during the FU and PTOP periods) will be used to calculate the monthly scores. All the data up to the Month 6 visit will be included in the primary analysis.

Despite the measures implemented to prevent missing data [described in protocol section 7.2.2.2.4], some missing data will occur in the daily measurements, possibly translating into missing monthly scores. In the next sections "missing data" will refer to missing monthly scores.

A summary of missing patterns can be presented for each treatment group as illustrated in Table 10.

Table 10 Example: Summary of patterns of missingness

Pattern	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	N (%)
1	X	X	X	X	X	X	xx (xx.x%)
2	X	X	X	X	X	-	xx (xx.x%)
3	X	X	X	-	X	-	xx (xx.x%)
4	X	X	X	X	-	-	xx (xx.x%)
5	X	X	X	-	-	-	xx (xx.x%)

15.2.3 Primary statistical analysis

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

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

The observed data cannot be used to distinguish between missing at random (MAR) and missing not at random (MNAR) missing data mechanisms [NRC Report 2010] and in incomplete-data settings a definitive MNAR analysis does not exist [Molenberghs 2004].

An analysis based on a pattern-mixture model will be performed using a MAR imputation in the placebo arm while using a MNAR approach in the lucerastat arm: missing data will be imputed applying a control-based multiple imputation (MI) assuming MNAR using the 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.

More specifically, the imputations will be performed in two steps: “intermittent” missing data (a monthly score is missing but one or more monthly score[s] is [are] available in following month[s]) will be first imputed by a Markov chain Monte Carlo method using a non-informative Jeffreys prior, thus creating 500 partially imputed “monotone” datasets; then, the remaining missing data will be imputed once in each of these 500 “monotone” imputed datasets using the CR approach leading finally to 500 complete imputed datasets. Imputed values will be restricted to remain in the clinically relevant range of [0, 10].

The two-step MI procedure will be implemented by the following SAS® code:

```
proc mi data=h nimpute=500 seed=32767 minimum = . . . . 0 0 0 0 0 0 maximum = . . . . 10
10 10 10 10 10 minmaxiter= 1000000out=h2;
 mcmc impute = monotone;
 var treatment sex ertstatus baseline y1 -- y6;
run;
proc mi data=h2 nimpute=1 seed=32767 minimum = . . . . 0 0 0 0 0 0 maximum = . . . . 10
10 10 10 10 10 minmaxiter=1000000out=h3;
 by _imputation_;
 class treatment sex ertstatus;
 var sex ertstatus bsl y1 -- y6;
 mnar model (y1 -- y6 / modelobs=(treatment="placebo"));
 monotone regression;
run;
```

An analysis of covariance (ANCOVA) model will then 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. This analysis will be implemented by the following SAS® code:

```
proc mixed;
 by _imputation_;
 class sex ertstatus treatment;
 model change = sex ertstatus bsl treatment;
 lsmeans treatment / cl pdiff;
run;
```

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 final estimate is the mean of the 500 per-imputation estimates and the final variance is the sum of the average within-imputation variance and (1 + 1 / 500) times the between-imputation variance [Rubin 1987]. From the final point estimate and variance, the 95% CI will be determined.

The results aggregation of the MI procedure after analysis of the 500 complete datasets will be implemented by the following SAS® code:

```
proc mianalyze data = <ods output lsmeans from proc mixed>;
 ods output parameterestimates=lsm_comb;
 by treatment;
 modeleffects estimate;
 stderr stderr;
run;

proc mianalyze data = <ods output diffs from proc mixed>;
 ods output parameterestimates=diff_comb;
 modeleffects estimate;
 stderr stderr;
run;
```

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.

15.2.4 Sensitivity analyses

In the event of discrepancies for the stratification factors between the IRT system and the eCRF, the analysis described in Section 15.2.3 will be repeated using the stratification factors as entered in the eCRF and considered as a sensitivity analysis of the main analysis.

The robustness of inferences from the primary endpoint analysis to deviations from its underlying modelling assumptions will be explored using several sensitivity analyses. Table 11 gives an overview of the planned sensitivity analyses that are described in this section.

Table 11 Summary of sensitivity analyses for missing data

Section	Variable	Imputation method	Analysis	Comment/purpose
15.2.3	Change from baseline to M6	MI (CR)	ANCOVA	Main analysis: MAR model in placebo arm, MNAR (CR) in lucerastat arm
15.2.4	Change from baseline to M6	MI (CR)	ANCOVA	Using the stratification factors as entered in the eCRF
15.2.4.1	Change from baseline to M6	MI (MAR)	ANCOVA	Imputation: MAR model Impact of departures from MNAR/CR assumptions
15.2.4.2	Change from baseline to M6	MI (J2R)	ANCOVA	Imputation: MAR model in placebo arm, MNAR (J2R) in lucerastat arm Impact of departures from MNAR/CR assumptions

M6: Month 6.
ANCOVA = Analysis of covariance; CR = copy reference; eCRF = electronic case report form; J2R = jump to reference, MAR = missing at random; MI = multiple imputation; MNAR = missing not at random.

15.2.4.1 Model-based analysis relying on the 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 15.2.3, 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.

The MI procedure will be implemented by the following SAS® code:

```
proc mi data=h nimpute=500 seed=32767 minimum = . . . . 0 0 0 0 0 0 maximum = . . . . 10
10 10 10 10 10 minmaxiter=1000000out=h2;
 mcmc impute = monotone;
 var treatment sex ertstatus baseline y1 -- y6;
run;
proc mi data=h2 nimpute=1 seed=32767 minimum = . . . . 0 0 0 0 0 0 maximum = . . . . 10
10 10 10 10 10 minmaxiter=1000000out=h3;
 by _imputation_;
 class treatment sex ertstatus;
 var treatment sex ertstatus baseline y1 -- y6;
 monotone regression;
run;
```

An ANCOVA model will then be used to analyze this endpoint on each imputed dataset as described in Section 15.2.3.

15.2.4.2 Model-based analysis relying on a different MNAR scenario

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.

The MI procedure will be implemented by the following SAS® code:

```
proc mi data=h6 nimpute=1 seed=32767 minimum = . . . . 0 maximum = . . . . 10
minmaxiter=1000000 out=h3;
 by _imputation_;
 class treatment sex ertstatus;
 var sex ertstatus bsl y6;
 mnar model (y6 / modelobs=(treatment="placebo"));
 monotone regression;
run;
```

An ANCOVA model will then be used to analyze this endpoint on each imputed dataset as described in Section 15.2.3.

15.2.5 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 methods and assumptions.

Table 12 below gives an overview of the planned supportive analyses that are described in this section.

Table 12 Summary of supportive analyses

Section	Variable	Imputation method	Analysis	Comment/purpose
15.2.3	Change from baseline to M6	MI (CR)	ANCOVA	Main analysis: MAR model in placebo arm, MNAR (copy reference) in lucerastat arm
15.2.5.1	Responder at M6	Missing = NR (SI)	CMH	Responder if score reduction from baseline at least 30% Missing at M6 = non-responder
15.2.5.2	Change from baseline to M6	MI (CR)	ANCOVA	Impact of not being treated (analysis on FAS)
15.2.5.3	Change from baseline to M6	MI (CR)	ANCOVA	Impact of protocol deviations (analysis on PPS)
15.2.5.4	Change from baseline to M6	MI (CR)	ANCOVA	Impact of use of significant rescue pain therapy Daily scores on days with use of any significant rescue pain therapy substituted by the worst score of subject during the double-blind treatment period
15.2.5.5	Change from baseline to M1, M2, ..., M6	None	MMRM	Mean score by treatment at each month Difference between treatment means at each month Characterization of response over time
15.2.5.6	Responder at M6 if x% reduction	Missing = NR (SI)	CMH	Impact of cut-off for responder definition: responder if score reduction from baseline at least x% (x = 20, 40, 50) instead of 30%
15.2.5.7	Percentage of subjects achieving a x% reduction at Month 6	Missing = NR SI)	Descriptive	Cumulative responder analysis in graph form
15.2.5.8	Improvement according to PGIC (PGIC-DS, PGIC-PS) at Month 6 visit	None	Description, ROC curve	Provides support for clinical relevance of the primary endpoint Using PGIC at Month 6 as an anchor, determines what would be the best cut-off on modified BPI-SF3 change (absolute change or percent change) based on data observed in the study population.
15.2.5.9	Improvement according to	None	Description, ROC curve	Provides support for clinical relevance of the primary endpoint

Section	Variable	Imputation method	Analysis	Comment/purpose
	change in PGIS-D (or PGIS-P) at Month 6 visit			Using change in PGIS-D at Month 6 as an anchor, determines what would be the best cut-off on modified BPI-SF3 change (absolute change or percent change) based on data observed in the study population Same approach applied separately for change in PGIS-P.
15.2.5.10	Change from baseline to M6	None	Descriptive	Investigates associations between change in neuropathic pain and changes in other FD manifestations (abdominal pain, diarrhea) Scatter plots by treatment group of change in modified BPI-SF3 score vs change in the considered symptom, Spearman correlation coefficients.

M1, M2, …, M6: Month 1, 2, 3, 4, 5, 6.
ANCOVA = Analysis of covariance; BPI-SF3 = Brief Pain Inventory-Short Form item 3; CMH = Cochran-Mantel-Haenszel test; CR = Copy Reference; FAS = Full analysis set; FD = Fabry disease; MAR = Missing at random; MI = multiple imputation; MNAR = Missing not at random; MMRM = mixed model for repeated measures; NR = non-responder; 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; PPS = Per-protocol analysis set; ROC = receiver operating characteristic; SI = single imputation.

15.2.5.1 Responder analysis

A cut-off of 30% in pain reduction was selected for this responder analysis because this 30% reduction in pain has been shown to represent important improvement in patients with neuropathic pain [Farrar 2001] and it is recommended that the percentages of patients responding with this degree of pain relief be reported in clinical trials of chronic pain treatments [Dworkin 2008]. Based on the evidence found in the literature in non-Fabry patients with neuropathic pain and regulatory guidelines [EMA 2007] (later replaced by [EMA 2017]), a cut-off of 30% was selected.

Using subject responses from the modified BPI-SF3, a response variable (yes/no) for the primary endpoint will be calculated based on a reduction of at least 30% from baseline to Month 6 in the modified BPI-SF3 score of "neuropathic pain at its worst in the last 24 hours".

Subjects who have a missing modified BPI-SF3 score at Month 6, for any reason, will be considered as non-responders, i.e., the worst possible outcome is assumed.

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.

This analysis will be implemented by the following SAS® code:

```
proc freq;
 tables sex*ertstatus*treatment*response / nocol nopercent cmh1
commonriskdiff(cl=newcombe);
run;
```

The Mantel-Haenszel risk (proportion) difference (lucerastat vs placebo) and corresponding 95% CI (stratified by sex and ERT treatment status at screening) will also be provided. The CIs are calculated using the stratified Newcombe method [Yan 2010].

15.2.5.2 Change from baseline to Month 6 on the FAS

If the FAS and mFAS differ by at least 5 subjects, an analysis on the FAS will be performed in order to assess the impact of not taking the study treatment. This analysis will be conducted as the main analysis.

15.2.5.3 Change from baseline to Month 6 on the PPS

An analysis on the PPS will be performed in order to assess the impact of protocol deviations on the assessment of the primary endpoint. This analysis will specifically address the issue of the lack of adherence to protocol or compliance with study medication until the intended EOS.

15.2.5.4 Change from baseline to Month 6 where data on days with use of any significant rescue pain therapy are substituted by a worst score

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 9.1 and the same analysis model as described in Section 15.2.3 will be applied.

15.2.5.5 Repeated-measures analyses of monthly modified BPI-SF3 scores up to Month 6

A mixed model for repeated measures (MMRM) will be used on the monthly change from baseline scores available at Month 1, Month 2, Month 3, Month 4, Month 5, and Month 6. The following terms will be included in the model: baseline modified BPI-SF3 score, treatment group, month, treatment group by month interaction and the two stratification factors sex and ERT treatment status at screening. An unstructured "UN" covariance structure will be primarily used for estimation of the correlation between responses

measured across the same subjects across multiple months (in the event of a convergence issue, a first order auto-regressive covariance structure will be used). The two study treatment groups will be compared at each month (with a primary interest in Month 6). The estimates of the differences in scores between treatment groups at each month will be reported with their 95% CIs. In addition, the adjusted means by treatment group at each month will be reported as estimates of the monthly scores across time.

This analysis will be implemented by the following SAS® code:

```
proc mixed;
 class subjid sex ertstatus treatment month;
 model change = sex ertstatus bsl treatment month treatment*month / ddfm=kr;
 repeated month / type = UN subject=subjid(treatment);
 lsmeans treatment*month;
 slice treatment*month / sliceby=month pdiff cl;
run;
```

15.2.5.6 Month 6 responders/non-responders analyses using different cut-offs of the percent reduction from the baseline score

A supportive analysis will derive the response at Month 6 by using cut-offs for response (20, 40, 50%) different from the 30% cut-off used in Section 15.2.5.1. The cut-offs of 40 and 50% have been mentioned in the literature [Dworkin 2008] as substantial change in pain. This analysis will be conducted as the analysis described in Section 15.2.5.1 (i.e., subjects with missing modified BPI-SF3 score at Month 6 will be considered as non-responders).

15.2.5.7 Empirical cumulative distribution function plots

Empirical cumulative distribution function (eCDF) plots will be provided for the absolute change from baseline to Month 6 and the percent change from baseline to Month 6 in the modified BPI-SF3 score by treatment group.

15.2.5.8 Determination of an anchor-based value for the change in modified BPI-SF3 when using PGIC as external anchor

In order to assess the clinical relevance in the context of the FD of the cut-off of 30% used in the responder analysis, the change in modified BPI-SF3 at Month 6 will be compared to the classification as responder/non-responder when using the PGIC at Month 6 visit as an external criterion. The methods used will be in line with previous publications in other diseases [Farrar 2010] and an FDA guidance for interpretation of patient-reported outcomes [McLeod 2011]. The two PGIC questionnaires (PGIC-DS, PGIC-PS) will be analyzed separately using the same approach.

These analyses will be conducted on the set of subjects with data available for both the change in modified BPI-SF3 at Month 6 and for the considered PGIC questionnaire at Month 6 visit. The analyses will be conducted on the two study treatment groups combined.

The change in modified BPI-SF3 at Month 6 will be expressed in two different ways: as absolute change from baseline and as percent change from baseline.

The polyserial and Spearman correlation coefficients between the change in modified BPI-SF3 at Month 6 and PGIC at Month 6 visit will be reported with their 95% CI.

This analysis will be implemented by the following SAS® code:

```
proc corr spearman fisher polyserial;
 with pgicchange;
 var percentchange;
run;
```

The confidence limits of the polyserial correlation coefficient will be derived as the coefficient ± 1.96 multiplied by its standard error. The confidence limits of the Spearman correlation coefficient will be derived using Fisher's z-transformation.

The correspondence between change in modified BPI-SF3 at Month 6 and the PGIC at Month 6 visit will be graphed by displaying the distribution (using box-plots) of modified BPI-SF3 at Month 6 within each PGIC level.

Three different response variables (yes/no), each corresponding to levels of improvement as characterized by the PGIC at Month 6 visit will be examined: "minimally improved" or better (PGIC = 1, 2 or 3); "much improved" or better (PGIC = 1 or 2); or "very much improved" (PGIC = 1).

For each of the three PGIC response variables and each of the two types of change in modified BPI-SF3 (absolute change or percent change), the empirical probability density function (ePDF) and the eCDF of each type of change in BPI-SF3 will be graphically represented for responders and non-responders according to the considered PGIC response variable. The ePDF will show on the y-axis, for each of the two categories (responder, non-responder) of the PGIC response variable the probability of observing a given modified BPI-SF3 change (kernel density estimated curves) vs the change in modified BPI-SF3 (on the x-axis). The eCDF will show, for each of the two categories (responder, non-responder) of the PGIC response variable, the total number of subjects, the cumulative proportion of subjects (on the y-axis) who reach a given modified BPI-SF3 change or less vs the change in modified BPI-SF3 (on the x-axis); horizontal lines at y = 50, 10, 25, 75, and 90% will illustrate the median change and the 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles of the change in modified BPI-SF3 for each response category. The ePDF and eCDF will also be presented using the original non-collapsed PGIC categories.

In addition, the empirical receiver operating characteristic (ROC) will be generated by calculating the sensitivity and 1-specificity in each 2 × 2 table using the number of subjects who achieve each observed level of change (or of percent change) in modified BPI-SF3 and the pre-selected level of improvement in PGIC as the outcome. For each empirical ROC curve, the area under the curve will be reported. Assuming an equal importance for

sensitivity and specificity, the optimal cut-off based on observed data will be defined as the point at the intersection of a 45° line with the ROC curve (i.e., the point minimizing the distance to the upper left corner). The corresponding sensitivity, specificity and agreement percentages (accuracy, positive predictive value, negative predictive value), will be reported.

15.2.5.9 Determination of an anchor-based value for the change in modified BPI-SF3 when using change in PGIS-D or change in PGIS-P as external anchor

In order to further assess the clinical relevance of the cut-off of 30% in the context of FD, the change in modified BPI-SF3 at Month 6 will be compared to the classification as responder/non-responder (improvement / no improvement [defined in Section 9.6.5]) when using the change in PGIS-D and the change in PGIS-P at Month 6 visit as external criterion.

These analyses will be conducted on the set of subjects with data available for both the change in modified BPI-SF3 at Month 6 and for the change in PGIS-D at Month 6. The analyses will be conducted on the two study treatment groups combined.

The change in modified BPI-SF3 at Month 6 will be expressed in two different ways: as absolute change from baseline and as percent change from baseline.

For each type of change in modified BPI-SF3, a box plot (including the individual data points) will represent the change in PGIS-D on the y-axis (ranging from –3 to +3) vs the change in modified BPI-SF3 on the x-axis and the corresponding polyserial and Spearman correlation coefficients will be provided.

Similarly as described in Section 15.2.5.8, ePDF and eCDF plots will be produced using the “improvement / no improvement” categories as defined in Sections 9.6.3 and 9.6.5 as well as each distinct category (i.e., 3-category improvement, 2-category improvement, 1-category improvement, no change, 1-category worsening, 2-category worsening, 3-category worsening).

The same series of analyses will be repeated on the change in PGIS-P.

For each of the two PGIS anchor scales, the following two tables will be provided [Table 13 and Table 14].

Table 13 Example: For patients who achieved a 1-category PGIS improvement from baseline at Month 6

		Baseline PGIS			
		Mild	Moderate	Severe	Total
Percent change in modified BPI-SF3 from baseline to month 6	n				
	Mean (SD)				
	10th Percentile (P10)				
	25th Percentile (Q1)				
	Median				
	75th Percentile (Q3)				
	90th Percentile (P90)				
	Min, Max				

BPI-SF3 = Brief Pain Inventory – Short Form item 3; PGIS = Patient Global Impression of Severity.

Table 14 Example: For patients who achieved a 2-category PGIS improvement from baseline at Month 6

		Baseline PGIS		
		Moderate	Severe	Total
Percent change in modified BPI-SF3 from baseline to Month 6	n			
	Mean (SD)			
	10th Percentile (P10)			
	25th Percentile (Q1)			
	Median			
	75th Percentile (Q3)			
	90th Percentile (P90)			
	Min, Max			

BPI-SF3 = Brief Pain Inventory – Short Form item 3; PGIS = Patient Global Impression of Severity.

15.2.5.10 Associations between change in neuropathic pain and other manifestations of FD, including plasma Gb3

15.2.5.10.1 Abdominal pain

The absolute change from baseline (Month 6 – baseline) in the NRS-11 score will be used to reflect the change in abdominal pain.

The analysis will be conducted on the set of subjects with a modified BPI-SF3 score available at Month 6 and non-missing change from baseline in the NRS-11 score. The analysis will be conducted on the two study treatment groups combined.

A scatter plot will represent the change from baseline in the NRS-11 score on the y-axis vs the absolute change in modified BPI-SF3 on the x-axis, and the corresponding Pearson and Spearman correlation coefficients will be provided with their 95% confidence limits.

This analysis will be implemented by the following SAS® code:

```
proc corr pearson spearman;
 with nrs11change;
 var percentchange;
run;
```

15.2.5.10.2 Diarrhea

The change from (Month 6 – baseline) in the number of days with at least one stool of a BSS consistency Type 6 or 7 will be used to reflect the change in diarrhea symptoms.

The analysis will follow the same approach as in Section 15.2.5.10.1.

15.2.5.10.3 Plasma Gb3

The analysis described in Section 15.2.5.10.1 will be repeated with the change from baseline to Month 6 in plasma Gb3.

15.2.6 Subgroup 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 subgroups considered in these analyses are defined in Section 6.

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 (LS-mean difference for lucerastat vs placebo) with its 95% CI for each level of each subgroup. It will be calculated as described for the primary analysis [Section 15.2.3]. 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.

15.3 Analysis of the secondary efficacy variables

The hypotheses for the secondary endpoints related to abdominal pain and plasma Gb3 are defined in the same way as described for the primary endpoint in Section 15.2.1.

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.

15.3.1 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

The same approach (CR MI + ANCOVA as main analysis with MAR MI and J2R MI + ANCOVA as sensitivity analyses) as described in Sections 15.2.3 and 15.2.4 will be used to analyze this endpoint on the mFAS-GIS. Subjects with the baseline value missing will be excluded from this analysis.

Subgroup analyses will be performed similarly to those described in Section 15.2.6 by including the respective subgroup and subgroup by treatment interaction in the ANCOVA.

The following supportive analyses will be performed:

- The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze a response variable (yes/no) defined as a reduction from baseline to Month 6 of at least 50% in the NRS-11 score.
- The same approach (MMRM) as described in Section 15.2.5.5 will be used to characterize the treatment effect over time.

Patterns of missingness will be summarized as described in Section 15.2.2. An eCDF plot of the absolute change from baseline to Month 6 by treatment arms will be provided as well.

Descriptive summary statistics of the absolute values and changes from baseline will be provided on the mFAS-GIS and the mFAS.

15.3.2 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

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. 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 BSS 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 CMH 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.

The three steps will be implemented using the following SAS® code.

```
/* 1) Produce standardized ranks for covariate BASE and response variable CHG – ensure to
impute ranks for CHG as described in the text below prior to this step */
* nplus1 option requests fractional ranks by using denominator n+1 where n is the strata-
specific sample size;
* ties=mean option requests midranks;
proc rank data = input nplus1 ties = mean out = ranks;
  by sex ert_status;
  var base chg;
run;

/* 2) Linear regression for each stratum on standardized ranks; output residuals */
proc reg data = ranks noprint;
  by sex ert_status;
  model chg = base;
  output out = residual r = resid;
run;

/* 3) Stratified mean score test, using values of residuals as scores, to compare
treatment groups */
proc freq data = residual;
  tables sex*ert_status*treatment*resid / noprint cmh2;
run;
```

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, derived via bootstrap (100,000 samples) using the bias-corrected method [Carpenter 2000] on the log-transformed win ratio. 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.

Bootstrapping will be implemented using the following SAS® code:

```
proc surveyselect data=ranks out=boot seed=197 method=urs samprate=1 reps=100000 outhits;
  strata treatment;
run;
```

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 the event 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 win ratio together with its 95% CI will also be derived within each subgroup defined in Section 6 (using the ranking for the main analysis described above), and presented in a forest plot. Bootstrapping will be performed separately for each subgroup to ensure constant numbers of subjects by treatment group within each bootstrap sample, using the following SAS® code:

```
proc surveyselect data=ranks out=boot seed=197 method=urs samprate=1 reps=100000 outhits;
  strata treatment subgroup;
run;
```

The following supportive analyses will be performed:

- The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze a response variable (yes/no) defined as a reduction from baseline to Month 6 of at least 50% in the number of days with at least one stool of a BSS consistency Type 6 or 7 in subjects who have diarrhea at baseline.
- The same approach (MMRM) as described in Section 15.2.5.5 will be used to characterize the treatment effect over time.

Patterns of missingness will be summarized as described in Section 15.2.2. An eCDF plot of the absolute change from baseline to Month 6 by treatment arms will be provided as well.

Descriptive summary statistics of the absolute values and changes from baseline will be provided on the mFAS-GIS and the mFAS.

15.3.3 Change from baseline to Month 6 in plasma Gb3

This absolute change 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 similarly to that described in Section 15.2.4.1. The imputation model includes the baseline value, the two stratification factors (sex and ERT treatment status at screening), Month 1, Month 3, Month 5, and Month 6 values and treatment group.

Subjects with the baseline value missing will be excluded from this analysis.

The MI procedure will be implemented by the following SAS® code:

```
proc mi data=h nimpute=500 seed=32767 min=. . . . 0 0 0 0 minmaxiter=1000000out=h2;
 mcmc impute = monotone;
 var treatment sex ertstatus baseline y1 y3 y5 y6;
run;
proc mi data=h2 nimpute=1 seed=32767 min=. . . . 0 0 0 0 minmaxiter=1000000out=h3;
 by _imputation_;
 class treatment sex ertstatus;
 var treatment sex ertstatus baseline y1 y3 y5 y6;
 monotone regression;
run;
```

The MAR assumption can be considered reasonable for this endpoint as it is an objective assessment and not patient-reported and therefore less likely to be related to reasons for study withdrawal. In addition, patients, physicians 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.

The analysis, within each imputed dataset, will be performed using an ANCOVA as described in Section 15.2.3.

Results from these analyses are combined using Rubin's methodology [Rubin 1987] implemented in SAS® PROC MIANALYZE.

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, control-based MI assuming MNAR using the CR and J2R approaches [described in Sections 15.2.3 and 15.2.4.2] will be applied.

Subgroup analyses will be performed similarly to those described in Section 15.2.6 by including the respective subgroup and subgroup by treatment interaction in the ANCOVA.

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 scheduled 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.

The main analysis described above (MI assuming MAR + ANCOVA) will be repeated (with the log-baseline as covariate) on log-transformed values (i.e., log[post-baseline] – log[baseline] = log[post-baseline / baseline]) leading via the back transformation $x -> ((e^x-1) \times 100)$ to treatment group results expressed as percent change from baseline and treatment effect estimate expressed as the ratio of the percent change between lucerastat and placebo. The log-transformation will only be applied after values have been imputed on the original scale, i.e., the imputation step is identical to the main analysis described above.

Patterns of missingness will be summarized as described in Section 15.2.2. An eCDF plot of the absolute change from baseline to Month 6 by treatment arms will be provided as well.

15.4 Analysis of other efficacy variables

15.4.1 Renal function endpoints

15.4.1.1 Subject eGFR slope from baseline to Month 6

A linear mixed model will be used to analyze this endpoint on the mFAS. The outcome variable will be the observed eGFR values measured from baseline up to the last available visit in the treatment period or PTOP. The following fixed effects will be included in the model: the two stratification factors (sex and ERT treatment status at screening), time (as a continuous variable), treatment group, and the treatment group by time interaction.

The model will include a random intercept and a random slope. An unstructured covariance structure will be used for estimation of correlation between random intercept and random slope. If this model fails to converge, a diagonal covariance structure will be used.

Time is derived as (eGFR measurement date – randomization date) * 12 / 365.25. Time will be set to 0 for the baseline value. The eGFR slope will be reported as mL/min/1.73m^2 per month.

The individual subject eGFR slopes will be derived as the random slope of each subject added to the mean slope in the respective treatment group.

Subjects with only one non-missing eGFR value from baseline up to Month 6 will not be included in the model and their eGFR slope will therefore be missing.

To assess the treatment effect, the estimate and the two-sided 95% CI will be calculated for the difference in the mean eGFR slope between lucerastat and placebo.

This analysis will be implemented by the following SAS® code:

```
proc mixed method=reml;
 class subjid sex ertstatus treatment;
 model egfr = sex ertstatus time treatment treatment*time / ddfm=kr solution cl;
 random intercept time / sub=subjid type=un gcorr solution group=treatment;
 ods output solutionf = fixed;
 ods output solutionr = random;
 estimate 'Lucerastat' time 1 treatment*time 1 0 / cl;
 estimate 'Placebo   ' time 1 treatment*time 0 1 / cl;
run;
```

15.4.1.2 Change from baseline to Month 6 in UACR

The ANCOVA after MI assuming MAR described in Section 15.3.3 will be used to analyze the change from baseline to Month 6 on the mFAS, on original values at baseline, Month 1, Month 3, Month 5, and Month 6.

Subjects with the baseline UACR value missing will be excluded from this analysis.

15.4.2 Echocardiography-based endpoints

An ANCOVA model will be used to analyze the change from baseline to Month 6 of each echocardiography parameter on the mFAS. 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.

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

This analysis will be implemented by the following SAS® code:

```
proc mixed;
 class sex ertstatus treatment;
 model chg = sex ertstatus bsl treatment;
 lsmeans treatment / cl pdiff;
run;
```

The analysis described above will be repeated on log-transformed values leading via the back-transformation $x \rightarrow (e^x-1) \times 100$ to treatment group results expressed as percent change from baseline and treatment effect estimate expressed as percent change relatively to placebo.

15.4.3 Pain medication endpoints based on daily entries in eDiary

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

The same approach (ANCOVA) as described in Section 15.4.2 will be used to analyze the overall mean weekly dose of opioid analgesics from baseline to Month 6 on the mFAS.

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

The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze this endpoint on the mFAS.

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

Due to the expected distribution of this endpoint (count data) and the varying observation times per subject, this endpoint will be analyzed by determining the annualized rate of days on significant rescue pain therapy using a negative binomial regression model adjusted for the stratification factors.

The observation time (in years; defined in Section 9.5.3) will be used as an offset variable in the model in order to account for varying lengths of the observation period for each subject.

The treatment effect will be expressed as a rate ratio between lucerastat and placebo and will be presented with its 95% CI.

Since days with missing eDiary entries are excluded from the calculations (not imputed), the ARRT is unbiased in the presence of missing data as long as the unobserved rate of days with significant rescue pain therapy over the missing entries is the same as that observed over the non-missing entries. This assumption is considered reasonable given that measures have been put in place to minimize the risk of missing data [see protocol section 7.2.2.2.4].

This analysis will be implemented by the following SAS® code:

```
proc genmod;
 class sex ertstatus treatment;

 model nbdays = sex ertstatus treatment / dist=negbin link=log offset=ltime;
 * ltime = log(observation time);
 lsmeans treatment / cl exp;
 estimate "lucerastat - placebo" treatment 1 -1;
run;
```

15.4.4 Clinical symptoms endpoints based on data collected at site visits

15.4.4.1 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")

The same approach (ANCOVA) as described in Section 15.4.2 will be used to analyze this endpoint on the mFAS. The same approach will also be used on the log-transformed data, leading (via the back transformation $x -> (e^x-1) \times 100$) to treatment group results expressed as percent change from baseline and treatment effect estimate expressed as percent change relatively to placebo.

15.4.4.2 Change from baseline to Month 6 in the total score of the subject's rating of item 9 of the BPI-SF

The same approach (ANCOVA) as described in Section 15.4.2 will be used to analyze this endpoint on the mFAS.

15.4.4.3 Change from baseline to Month 6 in the subject's rating of neuropathic pain severity as measured by the PGIS-P

The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze on the mFAS the proportion of subjects experiencing improvement.

15.4.4.4 Subject rating of change in neuropathic pain severity since study treatment start as measured by the PGIC-PS at Month 6

The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze on the mFAS each of the 3 response variables defined in Section 9.6.4.

15.4.4.5 Change from baseline to Month 6 in the subject's rating of disease severity as measured by the PGIS-D

The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze on the mFAS the proportion of subjects experiencing improvement.

15.4.4.6 Subject rating of change in disease severity since study treatment start as measured by the PGIC-DS at Month 6

The same approach (CMH test) as described in Section 15.2.5.1 will be used to analyze on the mFAS each of the 3 response variables defined in Section 9.6.6.

15.4.4.7 Change from baseline to Month 6 in the total score of the subject's rating of the CESD-R-20

The same approach (ANCOVA) as described in Section 15.4.2 will be used to analyze this endpoint on the mFAS.

The change from baseline to Month 6 in risk of depression will be compared between treatment groups using the CMH test stratified by sex and ERT treatment status at screening (treated vs not treated) at the two-sided significance level of alpha = 0.05.

Due to the ordinal nature of the change in risk, modified ridit scores will be used to obtain the Van Elteren extension of the Wilcoxon rank-sum test. This analysis will be implemented by the following SAS® code:

```
proc freq;
 tables sex*ertstatus*treatment*riskchange / nocol nopercent scores=modridit cmh2;
run;
```

The change from baseline to Month 6 in risk of depression will be further detailed within each treatment group by a shift table of the 5-level classification.

15.4.5 Time to treatment failure up to Month 6

The analysis of this endpoint will be performed using a two-sided stratified log-rank test, stratified by sex and ERT treatment status at screening. Kaplan-Meier estimates for the survival functions by treatment group will be plotted using Kaplan-Meier curves and will be tabulated by monthly intervals with their 95% CIs.

The treatment effect will be measured by means of a hazard ratio (and its 95% CI) calculated using a stratified Cox's proportional hazard model stratified by sex and ERT treatment status at screening.

This analysis will be implemented by the following SAS® code:

```
/* Kaplan Meier */
proc lifetest;
 strata treatment;
 id subjid;
 time time*censor(1);
run;

/* Stratified log-rank test */
proc lifetest;
 strata sex ertstatus / group = treatment;
 id subjid;
 time time*censor(1);
run;

/* Stratified Cox model */
proc phreg;
 class treatment (ref="placebo");
 model time*censor(1) = treatment / ties=exact rl;
 strata sex ertstatus;
run;
```

16 SAFETY ANALYSIS

16.1 Adverse events

All AEs and SAEs will be coded using the latest available version of MedDRA.

Analysis of AEs will be performed by counting subjects with a same MedDRA preferred term. Thus, a subject having experienced the same event (preferred term) more than once within the period of interest will be counted only once in the number of subjects with that event. A subject with several different events of the same SOC will be counted only once in the summary of events for each single SOC.

Summaries of AEs by treatment group will be presented by descending order of frequency in the active treatment group, i.e., SOC and preferred terms with the highest number of occurrences will appear first.

Treatment-emergent AEs will be summarized in a table by treatment group, SOC and preferred term displaying the total number of subjects exposed and the number (and percentage) of subjects reporting treatment-emergent AEs in total, by SOC and by preferred term within SOC.

Treatment-emergent AEs will also be summarized in a table by treatment group and preferred term.

The proportion of subjects who experienced treatment-emergent AEs will also be tabulated by SOC, preferred term, and maximal intensity. AEs with missing intensity will be considered as severe (the count of imputed AEs will be reported).

These 3 tables will be repeated counting only the treatment-emergent AEs (TEAEs) judged as related by the investigator.

Treatment-emergent SAEs and treatment-emergent SAEs judged as related by the investigator will be summarized similarly to TEAEs. A listing of all SAEs will be prepared on the SAF.

Treatment-emergent AEs leading to premature discontinuation of study treatment will be listed and summarized similarly to TEAEs.

For patients experiencing a treatment-emergent AE with fatal outcome, a summary table will present counts of patients by primary cause of death. A listing of all deaths will be prepared on the SCR.

16.2 Laboratory data

16.2.1 Change from baseline to each visit up to Month 6

For quantitative parameters, summaries of the observed value and change from baseline at each visit will be presented by treatment group, using the windowed times as described in Table 15. For categorical parameters (e.g., proteinuria), shift tables reflecting change from baseline will be presented by treatment group at each visit, using the windowed times as described in Table 15. Results of male reproductive hormones and of semen analyses will be listed.

Table 15 Time windows for all assessments up to EOT + 30 days

Visit	Treatment day (nominal value)	Lower limit treatment day	Upper limit treatment day
Month 1	30	2	45
Month 2	61	46	75
Month 3	91	76	106
Month 4	122	107	136
Month 5	152	137	167
Month 6	183	168	EOT + 30

Should more than one assessment fall within the same time window, then the closest value to the planned treatment day will be assigned to the visit. In the event of values that are equidistant to the planned treatment day, the latest assessment will be retained.

Where more than one assessment falls on the same day, the first available central assessment is used.

16.2.2 Treatment-emergent marked abnormalities

The number and percentage of subjects with treatment-emergent marked laboratory abnormalities will be tabulated by treatment group for each laboratory parameter for which marked abnormalities are defined in Table 16, Table 17 and Table 18. For a given parameter, percentages will be based on the number of subjects at risk: those not meeting

the criterion at baseline (or having a missing baseline value) and having at least one post-baseline value (not later than EOT + 30).

Marked abnormalities are classified as high (HH, HHH, HHHH flags) or low (LL, LLL, LLLL flags) based on values occurring above the higher limit or below the lower limit, respectively. It is possible that, for a given parameter, the same subject is counted as a high marked abnormality for an observed value and as a low marked abnormality for a different observed value. Only the most severe abnormality will be counted (e.g., a subject meeting the "HHH" criterion for a specific laboratory parameter will not be summarized under "HH" for this parameter).

Table 16 Marked abnormalities for hematology

Variable	Abnormality
Hemoglobin (g/L)	< 80 (LLL flag) < 100 (LL flag) > 20 above ULN or > 20 above baseline if baseline > ULN (HH flag) > 40 above ULN or > 40 above baseline if baseline > ULN (HHH flag)
Leukocytes (10^9/L)	< 2.0 (LLL flag) < 3.0 (LL flag) > 20.0 (HH flag) > 100.0 (HHH flag)
Neutrophils (10^9/L)	< 1.0 (LLL flag) < 1.5 (LL flag)
Lymphocytes (10^9/L)	< 0.5 (LLL flag) < 0.8 (LL flag) > 4.0 (HH flag) > 20.0 (HHH flag)
Eosinophils (10^9/L)	> 5.0 (HH flag)
Platelets (10^9/L)	< 50 (LLL flag) < 75 (LL flag) > 600 (HH flag) > 999 (HHH flag)

ULN = upper limit of normal range.

Table 17 Marked abnormalities for blood chemistry

Variable	Abnormality
ALT (U/L),	> 3 ULN (HH flag) > 5 ULN (HHH flag) > 8 ULN (HHHH flag)
AST (U/L)	> 3 ULN (HH flag) > 5 ULN (HHH flag) > 8 ULN (HHHH flag)
Alkaline phosphatase (U/L)	> 2.5 ULN (HH flag) > 5 ULN (HHH flag)
Bilirubin (µmol/L)	> 2 ULN (HH flag) > 5 ULN (HHH flag)
Creatinine (µmol/L)	> 1.5ULN or > 1.5 baseline if baseline > ULN (HH flag) > 3 ULN or > 3 baseline if baseline > ULN (HHH flag)
eGFR (mL/min/1.73 m^2)	< 15 (LLLL) < 30 (LLL) < 60 (LL)
Urea nitrogen (mmol/L)	> 2.5 ULN (HH flag) > 5 ULN (HHH flag)
Urate (µmol/L)	> 590 (HH flag) > 720 (HHH flag)
Albumin (g/L)	< 20 (LLL flag) < 30 (LL flag)
Glucose (mmol/L)	< 2.2 (LLL flag) < 3.0 (LL flag) > 8.9 (HH flag) > 13.9 (HHH flag)
Sodium (mmol/L)	< 130 (LL flag) > 150 (HH flag) > 155 (HHH flag)
Potassium (mmol/L)	< 3.0 (LLL flag) < 3.2 (LL flag) > 5.5 (HH flag) > 6.0 (HHH flag)
Calcium (mmol/L)	< 1.75 (LLL flag) < 2.0 (LL flag) > 2.9 (HH flag) > 3.1 (HHH flag)

ALT = aspartate aminotransferase; AST = alanine aminotransferase; BUN = blood urea nitrogen; eGFR = estimated glomerular filtration rate; ULN = upper limit of normal range.

Table 18 Marked abnormalities for urinalysis (dipstick analysis)

Variable	Abnormality
Bilirubin	3+ (HH flag)
Occult blood	3+ (HH flag)
Glucose	3+ (HH flag)
Ketones	3+ (HH flag)
Leukocyte esterase	3+ (HH flag)
Protein	3+ (HH flag)

Patient listings will also flag values without marked abnormalities but outside of the normal range as H (high; LB.LBNRIND = HIGH) or L (low; LB.LBNRIND = LOW). Results with LB.LBSTRESC available as < x.x (below x.x) will be displayed as < x.x in the patient listing but analyzed as x.x (equal to x.x). Results with LB.LBSTRESC available as > x.x (above x.x) will be displayed as > x.x in the patient listing but analyzed as x.x (equal to x.x).

Semen analysis data will be listed with a flag (L) for sperm concentrations corresponding to a decrease from baseline of 50% or more.

16.3 Vital signs

16.3.1 Change from baseline to each visit up to Month 6

Summaries of the observed value and change from baseline at each visit will be presented by treatment group, using the windowed times as described in Table 15.

16.3.2 Treatment-emergent marked abnormalities

The number and percentage of subjects with treatment-emergent marked abnormalities will be tabulated by treatment group for each vital sign variable. For blood pressures and heart rate, percentages will be based on the number of subjects at risk for the parameter: those not meeting the criterion at baseline (or with a missing baseline value) and with at least one post-baseline value. For weight, percentages will be based on the number of subjects at risk for this parameter: those with a baseline value and at least one post-baseline value (not later than EOT + 30).

Marked abnormalities are classified as high (H, HH) or low (L, LL) based on values occurring above the higher limit or below the lower limit, respectively. It is possible that, for a given parameter, the same subject is counted as a high marked abnormality for an observed value and as a low marked abnormality for a different observed value. Only the most severe abnormality will be counted (e.g., a subject meeting the “HH” criterion for a specific parameter will not be summarized under “H” for this parameter).

The definitions of marked abnormality used for SBP, DBP, HR, and body weight are presented in Table 19.

Table 19 Marked abnormalities for vital signs

Variable	Abnormality
SBP (mmHg)	< 90 (L flag) > 140 (H flag) > 180 (HH flag)
DBP (mmHg)	< 60 (L flag) > 90 (H flag) > 120 (HH flag)
HR (bpm)	< 45 (LL flag) < 50 (L flag) > 100 (H flag)
Weight (kg)	decrease from baseline > 10% (L flag) increase from baseline > 10% (H flag)

bpm = beats per minute; DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure.

16.4 12-lead ECGs

12-lead ECG parameters include HR (bpm), PR (ms), QRS (ms), QT (ms), QTcB (ms), QTcF (ms), and any morphological abnormalities as defined by the ECG provider.

16.4.1 Change from baseline to each visit up to Month 6

Summaries of the observed value and change from baseline at each visit will be presented by treatment group, using the windowed times as described in Table 15. In addition, summaries of the observed value and change from pre-dose at Month 1 (as recorded in the eCRF) to 2 hours and 4 hours post-dose at Month 1 visit will be presented by treatment group.

16.4.2 Treatment-emergent marked abnormalities

The number and percentage of subjects with treatment-emergent marked abnormalities will be tabulated by treatment group for each ECG variable. For a given parameter, percentages will be based on the number of subjects at risk: those not meeting the criterion at baseline (or with a missing baseline value) and with at least one post-baseline value (not later than EOT + 30).

Marked abnormalities are classified as high (H, HH, HHH) or low (L, LL) based on values occurring above the higher limit or below the lower limit, respectively. It is possible that, for a given parameter, the same subject is counted as a high marked abnormality for an observed value and as a low marked abnormality for a different observed value. Only the most severe abnormality will be counted (e.g., a subject meeting the “HH” criterion for a specific parameter will not be summarized under “H” for this parameter).

The definitions of marked abnormality used are presented in Table 20.

Table 20 Marked abnormalities for 12-lead ECGs

Parameter	Abnormality
HR (bpm)	< 45 (LL flag) < 50 (L flag) > 100 (H flag)
PR (ms)	> 200 (H flag) > 220 (HH flag) < 120 (L flag)
QRS (ms)	> 110 (H flag)
QTcB (ms)	> 450 (H flag) > 480 (HH flag) > 500 (HHH flag) < 340 (L flag)
QTcF (ms)	> 450 (H flag) > 480 (HH flag) > 500 (HHH flag) < 340 (L flag)

ECG = electrocardiogram; HR = heart rate; QTcB = QT corrected according to Bazett's formula; QTcF = QT corrected according to Fridericia's formula.

ECG findings reported by the ECG provider (EG.EGORRES where EG.EGCAT = FINDING) will be listed with a flag indicating ECG examinations with morphological abnormalities as defined by the ECG provider (where EGTEST = Interpretation and EG.EGORRES in [Abnormal ECG, probably non-significant, Abnormal ECG, possibly significant]).

17 ANALYSIS OF QUALITY OF LIFE ENDPOINTS

The same approach (ANCOVA) as described in Section 15.4.2 will be used to analyze on the mFAS the changes in the T-score of each of the 8 domains and the change in the T-score of each of the 2 component scores.

The health transition item (question 2: "Compared to one year ago, how would you rate your health in general now?") at baseline and Month 6 will be only listed.

18 ANALYSIS OF PHARMACOKINETIC ENDPOINTS

18.1 Assumptions

The following assumption is made:

- C_{max}, AUC_{τ}, and $t_{½}$ values are log-normally distributed [Julious 2000].

18.2 Calculation of PK endpoints

Plasma PK parameters will be determined by non-compartmental methods using Professional WinNonlin version 8.0 or higher, Pharsight Corporation, Mountain View, CA, USA and calculated on the basis of the actual blood sampling time points.

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.

λ_z represents the terminal elimination rate constant determined by log-linear regression analysis of the measured plasma concentrations in the terminal elimination phase.

The $t_{½}$ of lucerastat will be calculated as follows: $t_{½} = \ln 2 / \lambda_z$.

18.3 Descriptive analysis of PK endpoints

18.3.1 Listing of plasma concentrations

Plasma concentrations of lucerastat of the PK trough set (including Month 1 PK profile data when applicable) will be listed by subject (displaying the renal function level at screening) and by scheduled time point displaying actual times of blood sampling as well as elapsed actual times. Elapsed actual time in hours will be the number of hours elapsed from date and time of last study treatment intake to actual dates and times of sampling recorded in the (e)CRF.

18.3.2 Plasma concentrations

These concentrations will be summarized on the PK trough set (excluding Month 1 post-dose samples) and on the PK sub-study set by renal function level and overall per time point using number of subjects (n), arithmetic mean, minimum, median, maximum, SD, standard error (SE), and two-sided 95% CI of the mean. Concentrations reported as BLQ will be set to zero. If more than 50% of the values at a given time point are BLQ, no mean (including 95% CI), SD, and SE will be calculated for this time point.

18.3.3 PK parameters

All PK parameters (AUC_τ, C_{max}, t_{max}*, and $t_{½}$) of lucerastat will be listed by renal function. These parameters will be summarized by renal function level and overall with arithmetic mean, minimum, median, maximum, SD, SE, geometric mean, coefficient of variation between subjects (CV_b) in %, and 95% CIs of arithmetic and geometric means [statistical algorithms are detailed in Appendix B].

* For t_{max}, the geometric mean and its 95% CI and CV will not be calculated.

19 ANALYSIS OF BIOMARKERS OF FABRY DISEASE AND LUCERASTAT MECHANISM OF ACTION

Plasma lysoGb3, GlcCer, LacCer and urine Gb3 and lysoGb3 will be analyzed as described in Section 15.3.3 for plasma Gb3.

In addition, change from baseline (expressed as difference and expressed as percent change) will be descriptively analyzed at each time point.

20 CHANGES TO ANALYSES PLANNED IN THE STUDY PROTOCOL

20.1 Subgroup analyses

The study protocol lists the following subgroups related to pain medications in section 10.3.2.6:

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

The last two of these were combined into the following subgroup in Section 6 of this SAP:

- Chronic anti-epileptics/anti-depressants (yes, no).

Rationale for change: the "Background TCAs" subgroup was requested by the FDA during the interaction at the time of protocol design. However, very few subjects are on chronic TCA in this study and a clinical interpretation of such a subgroup analysis would not be possible. On the other hand, the use of chronic anti-epileptics and/or anti-depressants to treat neuropathic pain can be considered as a sign of severity and chronicity of neuropathic pain. Hence, the initial TCA subgroup has been replaced by a broader subgroup including any subject using at least one anti-epileptic or one anti-depressant on at least 21 days during the 4 weeks prior to randomization.

For the "Background pain medications (none vs monotherapy vs combinations)" subgroup, "monotherapy" was defined as the use of pain medications belonging to one single class of pain medications. In the SAP, "monotherapy" is defined as the use of one single pain medication, i.e., two pain medications from the same class taken for at least 21 days would not qualify as "monotherapy" but as "combination".

Rationale for change: at the time of protocol design, it was not expected that some subjects could be on more than one chronic anti-depressant or more than one chronic anti-epileptic. Keeping the original protocol definition would imply that a subject on two chronic anti-epileptics would be allocated to the monotherapy subgroup while a subject taking one anti-epileptic and one NSAID would be allocated to the combination therapy subgroup.

The word "background" was replaced by "chronic" for all subgroups related to pain medications.

Rationale for change: the protocol refers to "background pain medications" but omits to specify whether this refers to any background pain medication or chronic pain medication. It makes clinically more sense to assess the subjects with chronic pain medications as a

separate subgroup rather than mixing subjects with infrequent use of pain medications and those using pain medications on a daily basis.

It is also expected that defining chronic pain medications as pain medications used on at least 21 out of 28 days reflects the use of strong pain medications such as anti-epileptics and anti-depressants as opposed to "as needed" use of non-opioid analgesics such as NSAIDs or infrequent use of anti-epileptics or anti-depressants. Medications such as anti-epileptics or anti-depressants are expected to be used on a daily basis in line with their respective Summary of Product Characteristics / United States Prescribing Information. There is no evidence that an infrequent use of such pain medications has an effect on neuropathic pain.

In addition, the following subgroups were added in this SAP [see Section 6 for further details]:

- Fabry disease subtype (Classic vs Late onset).
- Sex-adjusted α-GalA activity (Male < 5%, Female < LLN vs. Male ≥ 5%, Female ≥ LLN).
- Mutation amenability to migalastat (Amenable vs Not amenable).
- Chronic pain medications (yes, no).

21 REFERENCES

[Carpenter 2000] Carpenter J, Bithell J. Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians. Statist. Med. 2000;19:1141–64.

[Carpenter 2013] Carpenter JR, Roger JH, Kenward MG. Analysis of longitudinal trials with protocol deviation: a framework for relevant, accessible assumptions, and inference via multiple imputation. J Biopharm Stat. 2013;23(6):1352–71.

[Cuzick 2005] Cuzick J. Forest plots and the interpretation of subgroups. Lancet. 2005;365:1308.

[Dworkin 2008] Dworkin RH, Turk DC, Wyrwich KW, Beaton D, Cleeland CS, Farrar JT, et al. Interpreting the clinical importance of treatment outcomes in chronic pain clinical trials: IMMPACT recommendations. J Pain. 2008;9(2):105–21.

[EMA 2007] European Medicines Agency. Guideline on clinical medicinal products intended for the treatment of neuropathic pain. CPMP/EWP/252/03 Rev.1. 24 January 2007 [accessed on 29 October 2020]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-medicinal-products-intended-treatment-neuropathic-pain_en.pdf

[EMA 2017] European Medicines Agency. Guideline on the clinical development of medicinal products intended for the treatment of pain. EMA/CHMP/970057/2011. 1 July 2017 [accessed on 29 October 2020]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-development-medicinal-products-intended-treatment-pain-first-version_en.pdf

[Farrar 2001] Farrar JT, Young JP, LaMoreaux L, Werth JL, Pool RM. Clinical importance of changes in chronic pain intensity measured on a 11-point numeric pain rating scale. Pain. 2001;94:149–58.

[Farrar 2010] Farrar JT, Pritchett YL, Robinson M, Prakash A, Chappell A. The clinical importance of changes in the 0 to 10 numeric rating scale for worst, least, and average pain intensity: analyses of data from clinical trials of duloxetine in pain disorders. J Pain. 2010;11(2):109–18.

[Julious 2000] Julious SA, Debarnot CA. Why are pharmacokinetic data summarized by arithmetic means? J Biopharm Stat. 2000 Feb;10(1):55–71.

[Koch 1982] Koch G, Amara I, Davis G, Gillings D. A review of some statistical methods for covariance analysis of categorical data. Biometrics. 1982;38(3):563–595.

[Koch 1990] Koch G, Carr G, Amara I, Stokes M, Uryniak T. Categorical data analysis. In: D. A. Berry, editor. Statistical methodology in the pharmaceutical sciences. Boca Raton: CRC Press; 2016. p. 403–488.

[McLeod 2011] McLeod L, Coon CD, Martin SA, Fehnel SE, Hays RD. Interpreting patient-reported outcome results: US FDA guidance and emerging methods, Expert Rev Pharmacoecon Outcomes Res. 2011;11(2):163–9.

[Molenberghs 2004] Molenberghs G, Thijs H, Jansen I, Beunckens C, Kenward MG, Mallinckrodt C, et al. Analyzing incomplete longitudinal clinical trial data. Biostatistics. 2004;5:445–64.

[NRC Report 2010] National Research Council, Panel on Handling Missing Data in Clinical Trials; Committee on National Statistics; Division of Behavioral and Social Sciences and Education. The prevention and treatment of missing data in clinical trials. The National Academies Press, Washington DC, USA.

[O'Kelly 2014] O'Kelly M, Ratitch B. Clinical trials with missing data: a guide for practitioners. Chichester, UK: John Wiley & Sons; 2014.

[Quade 1967] Quade D. Rank Analysis of Covariance. J Am Stat Assoc. 1967;62(320): 1187–1200.

[Radloff 1977] Radloff LS. The CES-D scale: A self report depression scale for research in the general population. Applied Psychological Measurements,1977;1:385–401.

[Rubin 1987] Rubin DB. Multiple imputation for nonresponse in surveys. New York, NY, USA: John Wiley & Sons; 1987.

[SAS Institute 2015] SAS Institute Inc. SAS/STAT® 14.1 User's Guide. SAS Institute Inc.; 2015.

[Schafer 1999] Schafer JL. Multiple imputation: a primer. Stat Methods Med Res. 1999;8:3–15.

[Stokes 2012] Stokes M, Davis C, Koch G. Chapter 7: Nonparametric methods. In: Stokes M, Davic C, Koch, G. Categorical data analysis using SAS. 3rd ed. Cary (NC): SAS Institute Inc; 2012. p. 175–188.

[Wang 2016] Wang D, Pocock S. A win ratio approach to comparing continuous non-normal outcomes in clinical trials. Pharm Stat. 2016;15(3):238–245.

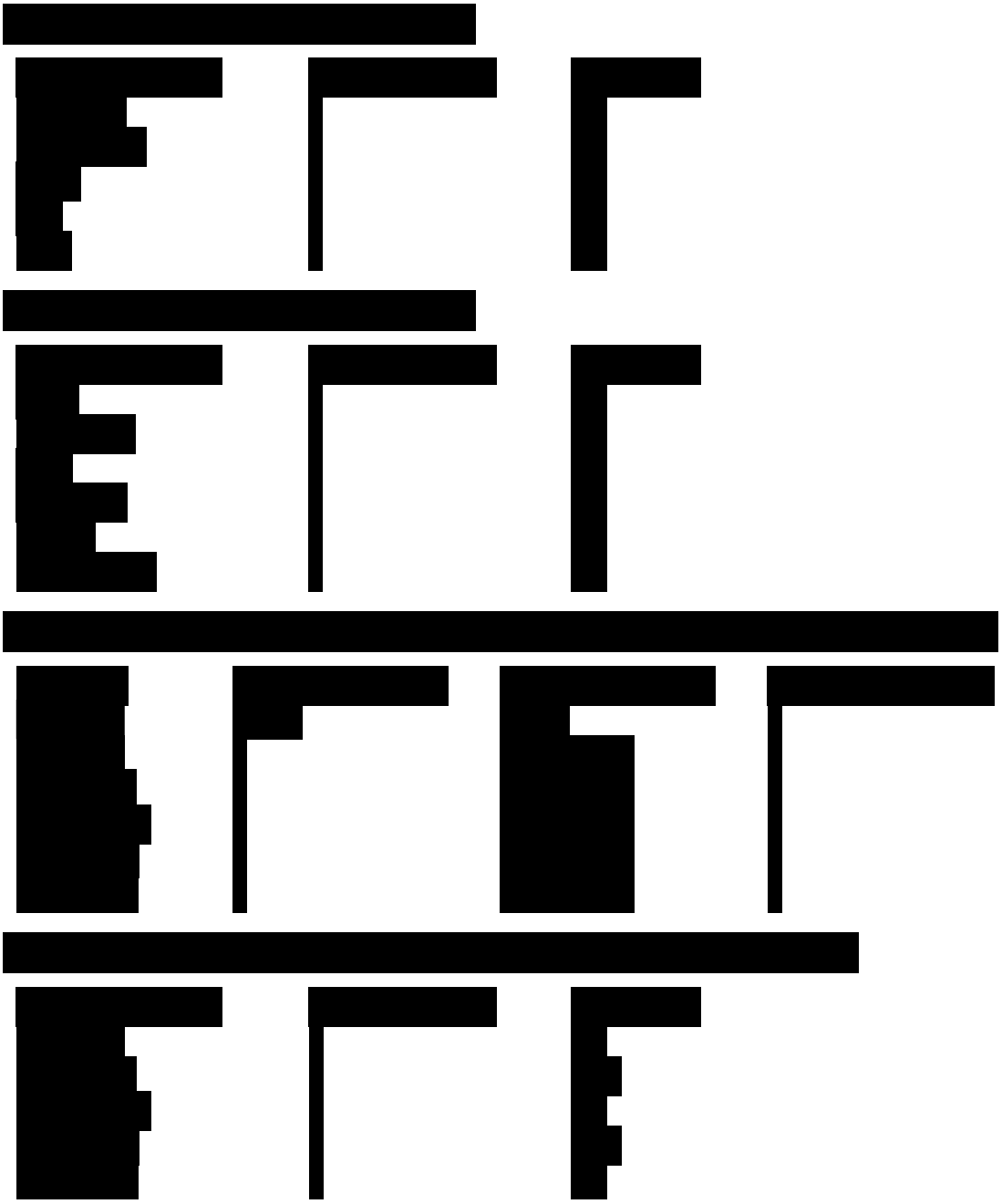
[Ware 2000] Ware JE, Kosinski M, Dewey JE. How to score version 2 of the SF-36® Health Survey. Lincoln, QualityMetric Incorporated.

[Yan 2010] Yan X, Gang Su X. Stratified Wilson and Newcombe Confidence Intervals for Multiple Binomial Proportions. Statistics in Biopharmaceutical Research 2010;2:329–35.

22 APPENDICES

A. Scoring of SF-36 Version 2

[redacted]



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[redacted]

[redacted]

B. Descriptive statistics used in analysis of PK endpoints

Statistic	Description
Mean	Arithmetic Average
Variance	Squared deviation of a random variable from its mean: $\frac{1}{n-1}\sum_{i=1n} (x_i - mean)^2$ with n the number of non-missing values.
SD	Standard Deviation: square root of the variance
CV%	Coefficient of variation: (SD/Mean)*100.
95% CI of the mean	Limits of the 95% CI for the mean defined as: $mean \pm t_{1-\frac{\propto}{2};n-1}\frac{SD}{\sqrt{n}}$ $t_{1-\alpha/2;n-1}$ is the (1 – α/2) percentile of the Student distribution (*t*-distribution) with n – 1 degrees of freedom, n = the number of observations. Thus, a 95% confidence level indicates that α = 0.05. Note: for n > 30, the *t*-distribution is close to the normal distribution.
Geometric Mean	The n^{th} root of the product of n non-missing values (note: all data must be positive): $(\prod_{i=1}^{n} x_i)^{\frac{1}{n}}$ The formula is identical to exp(mean of log-transformed data), a numerically more robust expression.
SD_{log}	SD of log-transformed data
CV% Geometric Mean	Coefficient of variation of the geometric mean: $100 * \sqrt{exp\left(\left(SD_{log}\right)^2\right) - 1}$
95% CI of Geometric Mean	Lower and upper limits of the 95% CI for the geometric mean.