STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER:

EC22-008

STUDY TITLE:

A Real World Evidence Study of Danish Fabry patients: a > 20-year Longitudinal Retrospective Analysis of Prospectively Collected Data.

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SPONSOR:

Amicus Therapeutics Ltd.
One Globeside, Fieldhouse Ln, Marlow SL7 1HZ, UK

SIGNATURE PAGE

This document has been prepared by:

Eva Krusinska, PhD, DSc
Biostatistics Consultant
Amicus Therapeutics

Signature Date

This document has been reviewed and accepted by:

Anette Heymann
Senior Medical Adviser, Nordics
Amicus Therapeutics

Signature Date

Biliana Veleva-Rotse
Director, Global Medical Affairs Fabry
Amicus Therapeutics

Signature Date

Caroline Kistorp
Professor, Chief Physician
Department of Hormone and Metabolism
Copenhagen University Hospital, Rigshospitalet

Signature Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition of Term
α-Gal A	α-galactosidase A
ACR	albumin-creatinine ratio
CI	confidence interval
ECG	electrocardiogram
ECHO	echocardiogram
eGFR	estimated glomerular filtration rate
$eGFR_{CKD-EPI}$	estimated glomerular filtration rate based on the Chronic Kidney Disease Epidemiology Collaboration equation
ERT	enzyme replacement therapy
ESRD	end-stage renal disease
FACE	Fabry-associated clinical event
FEV1	Forced exploratory volume after 1 second
FVC	Forced vital capacity
LVEF	left ventricular ejection fraction
LVMi	Left ventricular mass index
lyso-Gb_3	globotriaosylsphingosine
MCS	SF-36 Health Questionnaire Mental Component Score
MedDRA®	Medical Dictionary for Regulatory Activities
MMRM	mixed model for repeated measures
MRI	Magnetic resonance imaging
MWFS	mid-wall fractional shortening
NHP	Nottingham Health Profile
NT-proBNP	N-terminal pro b-type natriuretic peptide
PCS	SF-36 Health Questionnaire Physical Component Score
PRO	patient reported outcomes
SAE	serious adverse event
SAP	Statistical Analysis Plan
UA/NSTEMI	non-ST segment elevation myocardial infarction
UPCR	Urine Protein to Creatinine Ratio

Abbreviation	Definition of Term
WBC	White blood cell
WHO-DRUG	World Health Organization Drug Dictionary

1. INTRODUCTION

This document outlines the statistical methods to be implemented during the analyses of data collected within the scope of Amicus Therapeutics Protocol Number: EC22-008 "A Real World Evidence Study of Danish Fabry patients: a > 20-year Longitudinal Retrospective Analysis of Prospectively Collected Data", version 2 dated 24 October 2023.

The purpose of this SAP is to provide a framework in which answers to the protocol objectives may be achieved in a statistically rigorous fashion, without bias or analytical deficiencies. Specifically, this plan has the following purposes:

- To outline the specific types of analyses and presentations of data prospectively that will form the basis for conclusions.
- To explain in detail how the data will be handled and analyzed, adhering to commonly accepted standards and practices of biostatistical analysis in the pharmaceutical industry. Any deviations from these guidelines must be substantiated by sound statistical reasoning and documented in writing in the final study report.

2. OVERVIEW OF STUDY DESIGN

This is a retrospective study of prospectively collected patient data with the use of secondary patient data from both paper and electronic patient files. All patients are treatment naïve at time of diagnosis. Both migalastat and ERT will be studied retrospectively. Also untreated patients will be studied by assessment of relevant clinical and laboratory variables. The analysis will originate at the time of diagnosis.

The patients have been followed for more than 20 years from 2001 until December 2023. All identified patients with Fabry disease, whether receiving specific Fabry medication or not, have been offered annual or biannual thorough workups including examinations of Fabry associated organ function and symptoms. The study will be carried out at the Danish National Fabry Centre, Department of Endocrinology, Copenhagen University Hospital.

All patients from the Danish National Fabry Centre will be included. Subgrouping is aimed at depending on available numbers: non-amenable, amenable, classic, non-classic, males, females, treated including type of treatment or untreated.

2.1. Study Objectives

To investigate time to first Fabry associated clinical event (FACE) (cardiac, renal, cerebrovascular, including death due to FACE) with particular focus on migalastat clinical outcomes and treatment outcomes preceding migalastat therapy.

To investigate the incidence and prevalence of FACEs with respect to Fabry specific treatment, (migalastat, ERT) or no treatment.

To describe FACEs in accordance with different genotypic and phenotypic groups.

2.1.1. Primary objectives

To investigate Fabry associated clinical events (cardiac, renal and cerebrovascular) with particular focus on migalastat clinical outcomes from the start of treatment and treatment outcomes preceding migalastat therapy.

Details will be provided I the effectiveness analysis.

2.1.2. Secondary objectives

To investigate the incidence and prevalence of FACEs with respect to each cohort: migalastat, ERT, or no treatment.

To describe FACEs in accordance with different genotypic and phenotypic groups.

To investigate the incidence and time to a first fatal or non-fatal cardiac, renal, and cerebrovascular clinical event, separated by each category.

2.1.3. Exploratory objectives

To describe disease progression with focus on organ involvement.

3. STUDY ENDPOINTS

3.1. Primary Effectiveness Analysis

The primary endpoint is time to first FACE (cardiac, renal, or cerebrovascular events) ") since start of treatment for migalastat and or ERT and from time of diagnosis for untreated with particular focus on the added value of the recent addition of migalastat as a treatment option. A formal statistical analysis will be using the initial treatment cohort. Descriptive statistics will be calculated since each start of treatment (after each switch).

FACEs are defined as the following:

- Cardiac clinical events:
  - Myocardial infarction
  - New symptomatic arrhythmia requiring medication, direct current cardioversion, or interventional procedure (e.g., ablation, pacemaker, or defibrillator implantation)
  - Unstable angina defined by national practice guidelines and accompanied by electrocardiographic changes and non-ST segment elevation myocardial infarction [UA/NSTEMI]) and accompanied by electrocardiographic changes
  - Congestive heart failure requiring hospitalization
  - Any major cardiac medical procedure (e.g., valve replacement, stent-implantation, transplant, or persistent atrial fibrillation)
- Cerebrovascular clinical events:
  - Stroke (documented by a physician)
  - Transient ischemic attack (documented by a physician)
- Renal clinical events
  - Doubling of serum creatinine level from the start of analysis (2 consecutive values)
  - End-stage renal disease (ESRD) requiring long-term dialysis or transplantation
- Death due to FACEs

All FACEs will be analyzed together as a composite and separately in each category (cardiac, cerebrovascular, and renal). Death due to FACE will be analyzed under the category in which it occurred (cardiac, cerebrovascular, or renal).

3.2. Secondary Effectiveness Analysis

Secondary endpoints listed below will be assessed by evaluating migalastat-treated and untreated patients and/or migalastat-treated and ERT-treated patients:

- The prevalence of FACEs from each baseline (diagnosis, and start of treatment) in migalastat-treated, ERT-treated patients and untreated patients

- The incidence of FACEs from each baseline (diagnosis, and start of treatment in migalastat-treated, ERT-treated patients and untreated patients
- Time to the first cardiac, cerebrovascular, and renal clinical event separately by each specific category (including death in these categories) from baseline (diagnosis and start of each treatment) using Cox proportional hazards model and descriptive statistics.
- Incidence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories)
- Occurrence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories)
- Annualized rate of change in eGFR based on the Chronic Kidney Disease Epidemiology Collaboration equation (eGFR $_{CKD\text{-}EPI}$) over time in migalastat-treated, ERT, and untreated patients . Analysis will also be performed by eGFR category at baseline if the counts are sufficient.
- The prevalence and incidence of a clinically significant change in mGFR and/or eGFR (according to KDIGO guidelines rapid progression is defined as a sustained decline in eGFR of more than 5 ml/min/1.73 m2 /yr) from each baseline (diagnosis, and start of treatment) in migalastat-treated, ERT-treated patients and untreated patients.
- The prevalence and incidence of micro (> 30 mg/g creatinine) or macroalbuminuria (> 300) by urinary albumin/creatinine ratio (ACR) or urinary 24 h protein excretion from each baseline (diagnosis, and start of treatment) in migalastat-treated, ERT-treated patients and untreated patients.

The analysis for the primary effectiveness endpoint will be performed at the 2-sided significance level of 0.05 to compare migalastat-treated and untreated patients. All other comparisons will also be done at the 2-sided significance level of 0.05 with no adjustment for multiple comparisons.

3.2.1. Pharmacodynamic Endpoints

Pharmacodynamic endpoints will be assessed by evaluations comparing migalastat-treated and untreated patients and/or migalastat-treated and ERT-treated patients:

- Plasma lyso-Gb_3
- WBC α-Gal A enzyme activity in males
- Leukocyte and plasma Gb3, lyso-Gb3, and α-GAL A enzyme activity
- Neutralizing antibodies during the ERT treatment

3.2.2. Safety Endpoints

The design of the study is characterised by secondary use of data previously collected from healthcare professionals for other purposes.

For this study, the submission of suspected adverse reactions in the form of ICSRs is therefore not required.

All adverse events/reactions collected for the study should be recorded and summarised in the interim safety analysis and in the final study report unless the protocol provides for different reporting with a due justification.

SAEs

- Vital signs:
  - resting systolic blood pressure
  - resting diastolic blood pressure
- Body weight
- Overall survival among all patients enrolled, as assessed by recorded patient deaths from any cause
- Laboratory parameters:
  - serum creatinine
  - urine creatinine
  - urine protein
  - urine albumin
  - urine ACR
- ECGs
- ECHOs, including left ventricular mass index (LVMi), if medically indicated and requested by a physician
- Occurrence of male infertility
- Infusion related reactions

3.2.3. Exploratory Organ Specific Endpoints

Reflecting the programme of the annual clinical examinations and questionnaires of the Danish Fabry patients.

Lung capacity: clinically significant changes in spirometry measures of obstructive and restrictive lung function evaluated by body plethysmography.

- Forced expiratory volume after 1 second (FEV1)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

- Forced vital capacity (FVC)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

Central and peripheral neurological, assessments

- Standardized sweat test (hypo, normo, hyperhidrosis), standard unit of measure: mg pr. 30 minutes
- Autonomic nerve function (small fibre assessment))
- Presence of Fabry associated abnormalities by brain MRI

Standardized tilt-test (Presence of orthostatism – yes/no)

- Heart rate variability (Age-dependent – impaired vs not)
- Orthostatic blood pressure

Audiological assessments

- Audiogram (Impaired vs not)

Ophthalmological and dermatological assessments

- Presence of cornea verticillata
- Presence of angiokeratoma

Composite phenotypic score

- Mainz Severity Score Index (Composite score)

Cardiac function (evaluated by echocardiography)

- Left ventricular mass index (LVMi)
- Left ventricular ejection fraction (LVEF)
- Fractional shortening
- Left ventricular end diastolic and systolic volumes
- Mid-wall fractional shortening (MWFS)

- Left ventricular wall thickness
- NT-proBNP and high sensitive Troponin T – will be analysed from the Danish Fabry biobank.
- Global longitudinal strain if available

Renal function

- Laboratory parameters: measured glomerular filtration rate by 99Tc-DTPA or 51Cr-EDTA

Other renal function parameters are analyses under effectiveness.

Patient reported outcomes (PRO)

- SF-36 Health Questionnaire (Composite score)
- SF-36 Health Questionnaire Physical Component Score (PCS)
- SF-36 Health Questionnaire Mental Component Score (MCS)
- NHP-Health Profile (Daily living questionnaire – composite score)
- Major Depression Inventory (Composite score)

4. SAMPLE SIZE JUSTIFICATION

Approximately 115 patients with established Fabry Disease, estimated 2/3 female and 1/3 male.

Sample size was not determined statistically. All patients from Danish National Fabry Centre are included.

4.1. Randomization

Not applicable for this study.

4.2. Unblinding Plan

This is a retrospective, multicenter, observational study. No unblinding is necessary.

5. DEFINITION OF PATIENT POPULATIONS TO BE ANALYZED

5.1. Safety Population

The Safety Population will include all patients with Fabry disease who consented to participate and enrolled in the study. Patient disposition and reason for discontinuations will be presented in the Safety Population.

Patients who enter the study as untreated but switch to a treatment (migalastat or ERT) within 3 months from enrollment will be considered in analysis as initially belonging to a treatment group they switched to. Depending on an analysis as further discussed the group assignment might change in the case of a treatment switch.

5.2. Effectiveness Populations

5.2.1. Primary Effectiveness Population

The Primary-Effectiveness Population is defined as subsets of the migalastat-treated group, the untreated group, and ERT-treated group as comparators.

Patients who enter the study as untreated but switch to a treatment (migalastat or ERT) within 3 months from enrollment will be considered in analysis as initially belonging to a treatment group they switched to. Depending on an analysis as further discussed the group assignment might change in the case of a treatment switch.

All data collected will be utilized to establish the Primary Effectiveness Population for the analysis. Patients need to have a documented amenable variant to be included in the Primary Effectiveness Population and must meet criteria for receiving treatment for migalastat.

All patients from the Danish National Fabry Centre will be included, estimating the inclusion of 115 individuals (sex ratio [female:male] – 2:1). Pre-specified subgroups include: non-amenable/ amenable to migalastat, males/females.

5.2.2. Secondary Effectiveness Population

Secondary Effectiveness Population will include all patients regardless of the variant.

6. HANDLING OF DROPOUTS AND MISSING VALUES

6.1. Imputation of Dates

SAEs and FACEs

If onset date is completely missing, then the onset date is set to the date of enrollment unless end date is before the date of enrollment, in which case the onset date is set to 28 days prior to end date.

If (year is present and month and day are missing) or (year and day are present and month is missing):

- If year = year of enrollment, then set month and day to month and day of enrollment unless end date is before the date of enrollment, in which case the onset date is set to 28 days prior to end date.
- If year < year of enrollment, then set month and day to 31 December.
- If year > year of enrollment, then the following applies:
  - If year = year of first treatment switch, then set month and day to month and day of first treatment switch unless end date is before the date of treatment switch, in which case the onset date is set to 28 days prior to end date.
  - If there is no treatment switch, then set month and day to 1 January.

If month and year are present and day is missing:

- If year = year of enrollment and if month = month of enrollment, then set day to day of enrollment date unless end date is before the date of enrollment, in which case the onset date is set to 28 days prior to end date.
- If month < month of enrollment, then set day to last day of the month.
- If month > month of enrollment, then the following applies:
  - If year = year of first treatment switch and if month = month of first treatment switch, then set the day to the day of treatment switch unless end date is before the date of treatment switch, in which case the onset date is set to 28 days prior to end date.
  - If there is no treatment switch, then set day to first day of the month.

For all other cases, set onset date to the date of enrollment unless end date is before the date of enrollment, in which case the onset date is set to 28 days prior to end date.

End date will not be imputed.

Start and End of Therapy Prior to Study Enrollment

The following dates are essential for analysis: the end date of Fabry disease therapy prior to enrollment and the start date of ongoing Fabry therapy for the migalastat-treated patients and for the ERT-treated patients. These dates will be used to determine treatment duration prior to enrollment.

Prior to imputation of dates, the percentage of patients with completely missing start of therapy date, and separately with missing month will be calculated and presented in a table overall and by treatment group at enrollment.

Similarly, the percentage of patients with completely missing end of therapy date, and separately with missing month will be calculated and presented in a table overall and by treatment group at enrollment.

At least the year needs to be present to determine if the patients are eligible for inclusion in the Primary and Secondary Effectiveness Populations.

The following rules apply to the start date:

- If year is present and month and day are missing: set month and day to 1 July.
- If year and day are present and month is missing: set month to July.
- If year and month are present and day is missing: set day to the 15th of the month.

The following rules apply to the end date:

- If the end date is completely missing and the patient did not switch therapy, then the end date is imputed as the discontinuation date.
- If the end date is completely missing and the patient switched therapy, then the end date is imputed as the therapy switch date.
- If year is present and different from the year of the start date, and month and day are missing: set month and day to 1 July.
- If year is present and the same as the year of the start date, and month and day are missing: set month and day to 31 December.
- If year and day are present and month is missing: set month to July.
- If year and month are present and day is missing: set day to the 15th of the month.

Any partial dates will be displayed in data listings without and with imputation of missing days and/or months.

Treatment Switch

Prior to imputation of dates, the percentage of patients with completely missing switch of therapy date, and separately, the percentage of patients with missing month will be calculated and presented in a single table overall, and by treatment group at enrollment. Patients can change therapy more than once during the 20-year period. The percentages of patients with any completely missing switch of therapy dates or any missing therapy switch month will be presented. Additionally, a table with percentages of missing switch dates by group a patient switched to (migalastat-treated, ERT, untreated) will be presented.

The following rules apply to start date of new or switched therapy:

- If start date is completely missing: start date will be imputed as 30 days past enrollment date.

- If (year is present and month and day are missing) or (year and day are present and month is missing): set month and day to the date of the first closest visit, unless another switch occurred before it. In such a case, set the start date to the date of the previous visit.
- If year and month are present and day is missing: set day to the first day of the month unless during that month there was a visit, in which case the day will be set to the day of that visit.

The following rules apply to end date of new or switched therapy:

- If end date is completely missing: end date will not be imputed unless there is another treatment switch. In that case, the end date is imputed as a day before that next treatment switch.
- If (year is present and month and day are missing) or (year and day are present and month is missing): set month and day to the date of the last visit in that year, unless another switch occurred. In such a case, set the end date to a day before that next treatment switch.
- If year and month are present and day is missing: set day to the date of the last day of the month.

Any partial dates will be displayed in data listings without and with imputation of missing days and/or months.

Concomitant Medications

The following rules apply to start date:

- If start date is completely missing: start date will not be imputed.
- If (year is present and month and day are missing) or (year and day are present and month is missing): set month and day to 1 January.
- If year and month are present and day is missing: set day to first day of the month.

The following rules apply to end date:

- If end date is completely missing: end date will not be imputed.
- If (year is present and month and day are missing) or (year and day are present and month is missing): set month and day to 31 December.
- If year and month are present and day is missing: set day to last day of the month.

Any partial dates will be displayed in data listings without imputation of missing days and/or months (eg, MAR2011, 2009). No other imputation of missing data will be performed.

6.2. Missing Assessments

All data will be listed as collected. Descriptive statistics will be calculated on the data collected without replacement. For time to event, analysis data will be censored.

Missing and partial dates will be replaced as described in Section 6.1.

While it is possible that some patients will have missing or partial date information for SAEs or other safety data collections, analyses of the safety endpoints will include all patients in the Safety Population, unless specifically stated otherwise.

Missing data might be present for the analyses of the clinical laboratories and vital signs (eg, where the parameter was not measured at a required time points, which are the dates of treatment switch), and PROs. In many situations, there will often be other measurements of the parameter within a time frame that is applicable eg. during each treatment period. In such cases, the closest measurement to the switch time point within the treatment period will be used for analysis (Section 11). If such a measurement does not exist, then the patient will be excluded from the analysis of that variable at that timepoint. If 2 measurements are equidistant from the scheduled time point, the first one will be used.

Estimated GFR and LVMi data will be used as is and the actual time of the measurement will be utilized in the analysis. Analysis of LVMi will be exploratory.

7. STATISTICAL ANALYSIS CONSIDERATIONS

7.1. Conventions

Unless otherwise noted, data will be summarized in tabular format by treatment groups (ie, migalastat-treated, ERT-treated, and untreated patients where appropriate). Enrollment summaries will also include a total summary column.

The precision of original measurements will be maintained in summaries, when possible. Means, medians, and standard deviations will be presented with an increased level of precision; means and medians will be presented to 1 more decimal place than the raw data, and the standard deviations will be presented to 2 more decimal places than the raw data.

Summaries of continuous variables that have some values recorded using approximate values (eg, < or >) will use imputed values. The approximate values will be imputed using the closest exact value for that measurement. For tables where rounding is required, rounding will be done to the nearest round-off unit. For example, if the round-off unit is the ones place (ie, integers), values ≥ XX.5 will be rounded up to XX + 1 while values < XX.5 will be rounded down to XX.

Percentages will be based on available data and denominators will generally exclude missing values. For frequency counts of categorical variables, categories whose counts are zero will be displayed for the sake of completeness. For example, if none of the patients discontinue due to "lost to follow-up," this reason will be included in the table with a count of 0. Categories with zero counts will not have zero percentages displayed.

Unless otherwise specified, percentages will be calculated based on the number of patients specified by the appropriate population definition.

7.2. Standard Calculations

Variables requiring calculation will be derived using the following formulas:

- Days – Durations, expressed in days between 1 date (date1) and another later date (date2), are calculated using the following formula:
  duration in days = (date2 - date1) + 1
- Weeks – Durations, expressed in weeks between 1 date (date1) and another later date (date2), are calculated using the following formula:
  duration in weeks = (date2 - date1)/7
- Months – Durations, expressed in months between 1 date (date1) and another later date (date2), are calculated using the following formula:
  duration in months = (date2 - date1)/30.4
- Years – Durations, expressed in years between 1 date (date1) and another later date (date2), are calculated using the following formula:
  duration in years = (date2 - date1)/365.25
- Minutes – Durations, expressed in minutes between 1 time point (time1) and another later time point (time2), are calculated using the following formula:
  duration in minutes = (time2 - time1)/60

- Age at time of diagnosis – The patient's age is calculated as the number of years from the patient's date of birth to the date of enrollment into the study if a date of birth is available:

  Age at time of diagnosis = ([Enrollment Date - Date of Birth]/365.25)

- If only the year of birth is available, the age is calculated as follows:

  Age at time of diagnosis = Time of diagnosis Year – Birth Year

- Age at Therapy Start – The patient's age is calculated as the number of years from the patient's date of birth to the date of migalastat or ERT therapy start if a date of birth is available:

  Age at Therapy Start = ([Therapy Start Date - Date of Birth]/365.25)

- If only the year of birth is available, the age is calculated as follows:

  Age at Therapy Start = Therapy Start Year – Birth Year

Some patients will have eGFR calculated using a Modification of Diet in Renal Disease ($eGFR_{MDRD}$) equation recorded on the electronic case report form (eCRF), some will have glomerular filtration rate (GFR) calculated using the $eGFR_{CKD\text{-}EPI}$ equation recorded on the eCRF, some might have both recorded, and some might have data recorded from a different method. If possible, Estimated $GFR_{CKD\text{-}EPI}$ values will be recalculated for all patients, regardless if they have $eGFR_{CKD\text{-}EPI}$ recorded on eCRF or not.

The $eGFR_{CKD\text{-}EPI}$ equation was developed in an effort to create a more precise formula to estimate GFR from serum creatinine and other readily available clinical parameters, especially when actual GFR is > 60 mL/min per 1.73 m^2.

The CKD-EPI equation, expressed as a single equation, is:

$$GFR = 141 * \min(Scr/\kappa, 1)^{\alpha} * \max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018 \text{ [if female]} * 1.159 \text{ [if black]} * 1.808 \text{ [if Japanese]}$$

Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

Age at the time of measurement will be used for calculation.

All $eGFR_{CKD\text{-}EPI}$ will be calculated using the above formula (Levey, Stevens et al. 2009, Delgado et al 2022).

Time-to-event endpoints will be reported in months, where the number of months is derived as days to event divided by 30.4 (ie, 365.25/12).

7.3. Statistical Reporting

Statistical analyses will be reported using summary tables, figures, and data listings. Continuous variables will be summarized with counts, means, standard deviations, medians, minimums, and maximums. Categorical variables will be summarized by counts and by percentage of patients. For event data, i.e., SAEs and the exposure-adjusted incidence rates will be summarized as

counts of patients with a new event per 1000 person-years exposure, with a 95% confidence interval (CI) using Ulm's method (Ulm 1990).

Recurrent events will be presented for SAEs and each specific FACE and will be shown as a total count of each event per 1000 person-years.

A Cox proportional hazards model will be used for the summary of time to event data.

Tables, listings, and figures will be presented in rich text file (RTF) format. Upon completion, all SAS programs will be validated by an independent programmer. In addition, all programmed outputs will undergo a senior level statistical review. The validation process will be used to confirm that statistically valid methods have been implemented and that all data manipulations and calculations are accurate. Checks will be made to ensure accuracy and consistency with this plan, consistency within tables, and consistency between tables and corresponding data listings. Upon completion of validation and quality review procedures, all documentation will be collected and filed by the project statistician or designee.

7.4. Dictionaries

The Medical Dictionary for Regulatory Activities (MedDRA®) will be used to code AEs, investigations, medical/ surgical procedure, indication and medical history for system organ class and preferred terms using Version 23.0 or the latest available version. The enhanced version of the World Health Organization Drug Dictionary (WHO-DRUG, latest available version) will be used to code all medications (prior and concomitant) to anatomical therapeutic chemical and preferred drug names.

8. PATIENT ACCOUNTING AND DISPOSITION

8.1. Patient Accounting

Patient enrollment will be tabulated by treatment group (migalastat, ERT, or untreated) and overall. The summary will include the number and percentage of patients in the Safety Population, the Primary and Secondary Effectiveness Populations.

8.2. Patient Disposition

Patient disposition will be summarized by treatment group and overall and at the time of diagnosis, at the start of treatment, and the time of treatment switch. Other time points will be investigated if needed. In addition, frequency counts and percentages of patients' reported reasons for lack of follow up data will be summarized by treatment group and overall. Disposition will be presented in the Safety Population, the Primary and Secondary Effectiveness Populations.

A patient listing will be presented to describe treatment switches patient study completion, reason for discontinuation, primary cause of death, and other reasons specified.

9. DEMOGRAPHIC AND OTHER CHARACTERISTICS

Demographic data, enrollment and treatment start characteristics, and medical history (Fabry disease history, FACEs history, and other medical history) will be summarized using descriptive statistics by treatment group at baseline. These summaries will be based on the Safety Population, and the Primary and Secondary Effectiveness Populations. Fabry disease history, cardiac event history, cerebrovascular event history, and renal event history will be summarized as presented on eCRFs; other medical history will be coded (Section 7.4).

Those patients who are missing measurements of the variable being analyzed will not be included in the summary for that variable.

9.1. Demographics

Demographic variables collected prior to enrollment include date of birth, sex, race, and ethnicity. Age at baseline date of diagnosis for untreated patents and the date of start of the first treatment for treated patients), sex, race, ethnicity, pregnancy status for female patients and fertility status for male patients, will be summarized and reported.

For the Safety Population, data will be summarized at the time of diagnosis.

Age at the time of diagnosis (migalastat-treated versus untreated patients) and age at the start of the first treatment (migalastat-treated versus ERT-treated patients) will be analyzed as a continuous variable and will also be compared between groups using the categories: (< 12, 12 to < 18, 18 to < 65, ≥ 65). Sex, ethnicity, and race will be analyzed as categorical variables.

Patient height and weight will analyzed at the time of diagnosis and at the treatment start. Height and weight will be reported in metric units (height in cm and weight in kg) and will be analyzed as continuous variables.

9.2. Disease Characteristics

Documented variant of the gene encoding α-galactosidase A (*GLA*) will be presented as a summary and a listing.

Previous history of FACEs will also be summarized as both binary Yes/No variable and number and type of events.

Estimated GFR values will be summarized. eGFR values entered into eCRFs might have been measured by different methods. Therefore, they will be recalculated from creatinine to provide $GFR_{CKD\text{-}EPI}$ values. $eGFR_{CKD\text{-}EPI}$ values will be used to define categories for renal impairment.

Renal impairment will be categorized as Normal (GFR > 90 mL/min/1.73 m^2), Mild (GFR ≥ 60 to 90 mL/min/1.73 m^2), Moderate (GFR ≥ 30 to 60 mL/min/1.73 m^2), and Severe (GFR < 30) and analyzed as a categorical variable.

9.2.1. Medical History

The following medical history collected at enrollment will be presented: history of diabetes, dyslipidemia, hypertension, and other medical history reported (coded as per Section 7.4).

Duration of these diseases will also be presented for patients with a documented start and end date, and will be calculated as:

End date – start date + 1

Similarly, ECG results and ECHO collection (Yes/No) and overall interpretation (ie, normal, abnormal, not clinically significant, abnormal, potentially clinically significant) will be presented. Additionally, all available ECHO endpoints will be summarized.

10. CONCOMITANT MEDICATIONS

Concomitant medications will be defined as medications taken on or after the date of enrollment into the study and through the duration of the study.

If the medication stop date is before the enrollment date, such a medication is considered a previous medication and will not be analyzed, with exception of the previous ERT therapy and the previous migalastat treatment but will be listed.

For prior ERT or migalastat therapy, an overall prior exposure table will be presented by treatment group at baseline. If the stop date is missing or therapy is continuing, the date to calculate prior exposure will be the study enrollment date. A data listing will include dose and infusion frequency.

Concomitant medications will be coded according to the WHO-DRUG and will be summarized by treatment group using the Effectiveness and Safety Populations.

All reports in this section will provide the number of patients who received a concomitant medication by preferred drug name, Anatomical Therapeutic Chemical (ATC) class, and overall. A patient who received multiple agents within an ATC class will only be counted once for that class. Similarly, a patient who received a drug multiple times will only be counted once for the preferred drug name. Patients will be accounted into the group at the time of concomitant medication start date.

11. DEFINITION OF STUDY TIME POINTS

11.1. Definition of Diagnosis Date

Enrollment is the date on which the patient was diagnosed with the Fabry disease. For analysis of assessments which were not collected on the diagnosis date, the assessment closest to the diagnosis date will be utilized. If there are 2 assessments that are equidistant from the diagnosis date, then the assessment prior to diagnosis will be utilized.

11.2. Definition of the Migalastat or ERT Treatment Start or Treatment Switch

For analysis, if assessments were not collected on the treatment start date or a treatment switch date, then the assessment closest to the treatment start date will be utilized. If there are 2 assessments that are equidistant from the treatment start date, then the assessment prior to treatment start date will be utilized. Only assessments performed on that treatment will be used.

Treatment start date is used as the Analysis Start Date for comparing migalastat and ERT-treated patients. Diagnosis date is used as the Analysis Start Date for comparing migalastat and untreated patients.

12. SUBGROUP ANALYSIS

Subgroup analyses will be performed by forming subsets of patients from the Safety Population, the Primary and Secondary Effectiveness Populations such as:

- Age (≤ 40, > 40 years). For the Safety Population, enrollment date will be used to determine a patient's age. For the Effectiveness Population, the age will be calculated utilizing Analysis Start Date, ie, diagnosis date for migalastat-treated versus untreated group comparison and start of treatment for migalastat-treated versus ERT-treated comparison.
- Sex (female versus male)
- Renal impairment (Normal [GFR > 90 mL/min/1.73 m^2], Mild [GFR ≥ 60 to 90 mL/min/1.73 m^2], Moderate [GFR ≥ 30 to 60 mL/min/1.73 m^2], Severe [GFR< 30 mL/min/1.73 m^2],). For the Safety Population, enrollment date will be used to determine patient' s eGFR. For the Primary and Secondary Effectiveness Populations, the eGFR will be calculated utilizing Analysis Start Date, ie, enrollment date for migalastat-treated versus untreated group comparison and start of treatment for migalastat-treated versus ERT-treated comparison.
- Proteinuria categories based on UPCR (< 0.5 g/g, 0.5 to 1.0 g/g, >1.0 g/g)
- Classic phenotype in males versus non-classic phenotype (all others: males and females). In males, the classic phenotype of Fabry disease is determined by residual α-Gal A enzyme activity that is minimal or absent (ie, less than 5% of mean normal) and typified by earlier age of onset and more severe symptoms with multiple organ involvement, whereas the non-classic phenotype has residual α-Gal A activity and a later onset, but also is associated with significant disease progression. Use of α-Gal A in the determination of phenotype is applicable to treatment-naïve males only.
- Treatment-naïve ERT-treated versus treatment-naïve migalastat-treated. Treatment-naïve ERT patients are Fabry treatment-naïve patients at the start of ERT. Treatment-naïve migalastat patients are Fabry treatment-naïve patients at the start of migalastat.
- Male patients who have enzyme activity assessment at baseline

For some of the subgroups, only descriptive statistics will be provided due to a small subgroup size.

13. ANALYSIS OF EFFECTIVENESS

13.1. Primary Endpoint

The comparison of interest is between migalastat-treated and untreated patients in the Primary and Secondary Effectiveness Populations. The analysis for the primary effectiveness endpoint will be performed at the 2-sided significance level of 0.05. This analysis will be performed when enough data are available.

Clinical effectiveness will be based on FACEs of cardiac, cerebrovascular, and renal events, and deaths due to FACEs. A composite event is defined as the occurrence of either cardiac, cerebrovascular, or renal clinical event, and death due to FACEs.

Subject matter experts will adjudicate all reports of FACEs. For all summary analyses, the adjudicated decisions will be used if they differ from the initial report. Both original and adjudicated decisions will be reported in listings. Only adjudicated decisions will be used for analyses.

Analyses will be performed in the Primary and Secondary Effectiveness Population.

The analyses described further in Section **Error! Reference source not found.** through Section **Error! Reference source not found.** will be performed for the endpoint of all FACEs combined and separately for all specific FACEs categories listed above (including deaths in these categories) as supportive analyses. A separate analysis of all deaths due to FACEs will also be conducted.

13.1.1. Cox Proportional Analysis for Fabry-associated Clinical Events

The time to the first event is defined as time from the Analysis Start Date to the first composite FACE (primary interest), and to the first cardiac, cerebrovascular, renal clinical event, or death due to FACEs. For the primary analysis, patients who complete the study without an event, are lost to follow-up, discontinued the study, started dialysis, had a kidney or heart transplant, or after 30 days they changed/switched therapy (migalastat group) or after they start therapy (untreated group) will be censored. Describing effectiveness in migalastat treated population is of the main interest.

Treatment Switch

The primary analysis will address events that occur on therapy from the Analysis Start Date up until 30 days after the patient stops therapy or switches therapy. The events that happen afterwards will be excluded. For the patients untreated at time of diagnosis, the primary analysis will address events from the time of diagnosis until the patient starts a therapy. The events that happen afterwards will be excluded. An additional analysis will be performed:

1. An analysis that will address events that occur on therapy from the time of Analysis Start Date up until the patient stops therapy or patient switches therapy. For the untreated patients, the analysis will address assessments from the time of diagnosis until the patient starts a therapy. The events that happen afterwards will be excluded.

Primary Analysis Method

The ratio of the hazards of treatment groups will be checked to ensure that the hazard ratios are constant over time. Secondly, the assumption that each variable in the model makes a linear contribution will be checked.

The covariates will include:

- sex
- age at the Analysis Start Date as a continuous variable
- $eGFR_{CKD\text{-}EPI}$ at the Analysis Start Date as a continuous variable
- LVMi at the Analysis Start Date
- previous history of cardiac, cerebrovascular, and renal clinical events (composite event) as related to Analysis Start Date
- Length of treatment for Fabry disease

If the measurements are not available, measurements prior to and closest to the Analysis Start Date will be used. In case that more than 50% of values are missing for a given covariate, it will be dropped from the analysis.

The estimate of hazard ratio for migalastat-treated/untreated along with the associated 95% CI will be calculated.

A sensitivity analyses will be done using Kaplan-Meier approach (log-rank test).

The analysis between migalastat and ERT will be performed like the analysis above for the comparison between migalastat treated and untreated patients. The origination of analysis will be start of treatment.

Because patients could switch treatment many times during the 20-year period, the time to the first event within each treatment period will be calculated from each switch date and descriptive statistics will be presented. Please note that each patient could be counted more than once in this analysis. Two options will be used for these analyses. The first analysis will address events that occur on therapy from the Analysis Start Date up until 30 days after the patient stops therapy or from the switch date to the date of a subsequent switch or stopping therapy. The second analysis will address events that occur on therapy from the Analysis Start Date up until the patient stops therapy or switches therapy or from the switch date to the date of a subsequent switch or stopping therapy.

13.1.2. Interval Based Cox Proportional Analysis for Fabry-associated Clinical Events

In the interval-based time to event analysis will be used with 3 time-dependent variables indicating whether or not a patient is on migalastat, ERT, or untreated at time *t*. A patient may be in 1 or more treatment groups and a patient may be both left and right censored. For example, a patient that begins migalastat, stops at Month 9, begins ERT at Month 24, and then dies at Month 26 will be represented 3 times in the design matrix; the first for migalastat being right censored at Month 9, the second being for ERT left censored at Month 24 and right censored at Month 26, and finally, for untreated, being right censored at Month 9 and having an event at Month 24.

13.2. Secondary Endpoint: Prevalence of FACEs

Treatment Switch

The first analysis will address events that occur on therapy from the Analysis Start Date up until 30 days after the patient stops therapy or from the switch date to the date of a subsequent switch or stopping therapy. The second analysis will address events that occur on therapy from the Analysis Start Date up until the patient stops therapy or switches therapy or from the switch date to the date of a subsequent switch or stopping therapy.

Analysis Method

The prevalence of FACEs is calculated as a ratio of patients with at least one FACE on a given therapy divided by the number of patients on that therapy. If a patient starts dialysis or receives a kidney or heart transplant, the events afterwards will be excluded.

13.3. Secondary Endpoint: Incidence of FACEs

As this is an observational study, patients may switch or stop therapies. If a patient starts dialysis or receives a kidney or heart transplant, the events afterwards will be excluded.

The analysis of the incidence rate (number of patients with at least 1 event per 1000 person-years) will be performed on the Primary and Secondary Effectiveness Populations.

Treatment Switch

The analysis will address events that occur on migalastat or ERT therapy from the Analysis Start Date or switch date up until 30 days after the patient stops therapy or switches therapy. For the patients untreated at enrollment to the study, the analysis will address events from the time of diagnosis until the patient starts a therapy or during periods that they are untreated after a switch.

The second analysis will address events that occur on migalastat or ERT therapy from the Analysis Start Date or switch date. For the patients untreated at enrollment to the study, the analysis will address events from the time of diagnosis until the patient starts a therapy or during periods that they are untreated after a switch.

Analysis Methods

The calculation of the incidence rate (number of events per 1000 person-years) will be performed on the Primary and Secondary Effectiveness Populations.

13.4. Secondary Endpoint: Time to first FACE by Category

Time to the first cardiac, cerebrovascular, and renal clinical event separately by each specific category (including death in these categories) from start of treatment or time of diagnosis will be analysed in the same way as the primary endpoint - composite FACEs (section 13.1).

13.5. Secondary Endpoint: Incidence of FACEs by Category

Incidence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories) will be analysed as described in Section 13.2 for the composite FACEs.

13.6. Secondary Endpoint: Prevalence of FACEs by Category

Prevalence of cardiac, cerebrovascular, and renal clinical events separately by each specific category (including death in these categories) will be analysed as in Section 13.3 for the composite FACEs.

13.7. Secondary Endpoint: Estimated Glomerular Filtration Rate to Compare Migalastat-treated, ERT Treated, and Untreated Patients

Estimated GFR will be summarized using descriptive statistics by treatment at diagnosis date. Descriptive statistics will be displayed for change from Analysis Start Date (time of diagnosis or treatment start) to e.g. time points such as the date of the first treatment switch, date of the treatment to migalastat or any other time points of interest.

For dialysis and transplant patients, the Analysis Start Date for eGFR will be reset to the last value before dialysis start or before transplantation and used thereafter.

Analysis Methods

Data will be listed. Listings will contain flags indicating therapy change. Spaghetti plots will indicate what treatment group a patient was on at the time of measurement.

Longitudinal analysis using Mixed Model for Repeated Measures (MMRM) will be used to analyze data over time. Trends over time using adjusted means will be plotted. Missing post-Analysis Start Date measurements will not be imputed as MMRM method allows for missing values. This analysis only accounts for changes over time regardless of therapy.

Covariates are:

- sex
- enrollment age as a continuous variable
- enrollment eGFR as a continuous variable
- proteinuria based on the UPCR value at enrollment
- LVMi at enrollment
- history of cardiac, cerebrovascular, and renal clinical events (a binary endpoint based on the composite event) prior to enrollment
- length of treatment for Fabry disease

If the measurements are not available, measurements prior to and closest to the Analysis Start Date will be used. In case that more than 50% of values are missing for a given covariate, it will be dropped from the analysis.

Additionally, the annualized rate of eGFR change will be calculated as the slope of the individual patients' regression equations over time. The annualized rate of change will be analyzed using t-test for migalastat-treated versus untreated patients. This analysis only accounts for changes over time regardless of therapy.

An alternative analysis will only utilize data from periods on migalastat treatment and separately on the ERT treatment, and untreated periods.

13.8. Secondary Endpoints: Prevalence and Incidence of Clinically Significant change in mGFR or eGFR

The prevalence and incidence of a clinically significant change in mGFR and/or eGFR (according to KDIGO guidelines rapid progression is defined as a sustained decline in eGFR of more than 5 ml/min/1.73 m2 /yr) from each baseline (diagnosis and start of treatment) to compare between migalastat-treated, ERT-treated patients and untreated patients will be analyzed using same methodology as for incidence and prevalence of FACEs.

13.9. Secondary Endpoint: Prevalence and Incidence of micro – or Macroalbuminuria

The prevalence and incidence of micro (> 30 mg/g creatinine) or macroalbuminuria (> 300) by urinary albumin/creatinine ratio (ACR) or urinary 24 h protein excretion from each baseline (diagnosis, and start of treatment) to compare between migalastat-treated, ERT-treated patients and untreated patients will be analyzed using same methodology as for incidence and prevalence of FACEs.

Analysis by ACR categories will be performed if enough data are available.

14. PHARMACODYNAMIC PARAMETERS

Pharmacodynamic parameters will be analyzed in the Effectiveness Population. Analyses of PD assessments will focus on WBC α-Gal A enzyme activity and plasma lyso-Gb_3, Urinary and plasma Gb3, lyso-Gb3, α-GAL A enzyme activity, and Neutralizing antibodies with ERT.

Data will be summarized using descriptive statistics will be displayed for change from Analysis Start Date (time of diagnosis or treatment start) to e.g. time points such as the date of the first treatment switch, date of the treatment to migalastat or any other time points of interest.

Analysis Methods

Data will be listed. Listings will contain flags indicating therapy change. Spaghetti plots over time for WBC α-Gal A enzyme activity in males and plasma lyso-Gb_3 for individual patients will be produced. Spaghetti plots will indicate what treatment group a patient was on at the time of measurement.

The change from the Analysis Start Date for a parameter will be defined as the difference between that measurement and the measurements at time points such as the date of the first treatment switch, date of the treatment to migalastat or any other time points of interest.

15. SAFETY ANALYSIS

All patients in the Safety Population will be included in all safety summaries. Safety analyses will also be conducted in safety Population.

15.1. Approach to Safety Analyses

As this is an observational study, patients may switch or stop therapies. In the Safety Population, analysis will present results based on the actual exposure, ie, treatment group at the time of the event (migalastat, ERT, or untreated). As patients can stop or switch therapy while enrolled in the study, they might be counted more than once in the analysis.

Serious adverse events will be analysed as described in Section 13 for the composite FACEs.

15.2. Serious Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product or device that does not necessarily have a causal relation between the product and the event. An AE can therefore be any unfavorable or unintended sign (including abnormal laboratory finding) or symptom temporally associated with a medicinal product, whether or not related to the medicinal product.

For this study, SAEs are of most importance and will be summarized in the analysis. An SAE is any untoward medical occurrence which:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongs hospitalization
- Results in persistent or permanent disability or incapacitation
- Results in congenital anomaly or birth defect
- Any important medical event that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the definition above, will also be reported as an SAE.

In the Safety Population, the following will be summarized for SAE's for each treatment group: migalastat, ERT, untreated at the time of event:

- Reason for seriousness
- Assessment of causality in relation to current treatment (Definite, Probable, Possible, Unlikely, Unrelated, Untreated at time of event)
- Maximal intensity (Mild, Moderate, Severe)
- Outcome (Ongoing, Resolved, Resolved with sequelae, Fatal, Unknown)
- Event associated with underlying illness (Yes/No)
- Action taken (Discontinued Yes/No)

In addition, a data listing will be provided for all SAEs.

15.3. Incidence of SAEs

Incidence of SAEs is based on actual exposure. The exposure is calculated as per Section 15.5. The analysis of the incidence rate (number of patients with at least 1 event per 1000 person-years) will be performed.

15.4. Overall Survival

To assess the overall survival among all patients, a Cox proportional hazards model will be used, provided that the assumptions of Cox proportional hazard are met. The ratio of the hazards of treatment groups will be checked to ensure that the hazard ratios are constant over time. Secondly, the assumption that each variable in the model makes a linear contribution will be checked. Otherwise, Kaplan-Meier log-rank test will be used for this analysis.

Overall survival is defined as time from enrollment into the study until death due to any cause (event).

Covariates will include:

- sex
- enrollment age as a continuous variable
- enrollment eGFR as a continuous variable
- LVMi at enrollment
- time on treatment (ERT or migalastat overall)
- history of cardiac, cerebrovascular, and renal clinical events (a binary endpoint based on the composite event) prior to enrollment
- length of therapy for Fabry disease

If the measurements are not available, measurements prior to and closest to the Analysis Start Date will be used. In case that more than 50% of values are missing for a given covariate, it will be dropped from the analysis.

In the Safety Population where patients are analyzed based on the actual exposure, a Cox model run similarly to the analysis in Section 13.1.2 will be used but with 3 time-dependent variables indicating whether or not a patient is on migalastat, ERT, or untreated at time t. A patient may be in 1 or more treatment groups and a patient may be both left and right censored. For example, a patient that begins migalastat, stops at Month 3, begins ERT at Month 6, and then dies at Month 9 will be represented 3 times in the design matrix; the first for migalastat being right censored at Month 3, the second being for ERT left censored at Month 3 and right censored at Month 6, and finally, for untreated, being right censored at Month 6 and having an event at Month 9.

The estimate of hazard ratio for migalastat/ERT and migalastat/untreated along with the associated 95% CI will be calculated.

15.5. Extent of Study Drug Exposure

Study treatment exposure will be summarized using the Safety Population.

Analyses for the extent of exposure will focus on the duration of each respective treatment (migalastat, ERT, or untreated). Duration will be defined as:

Dur = End date of treatment – start date of treatment +1

Breaks in therapy or discontinuation in exposure as well as switching therapy will be accounted in the calculations. Missing dates will be imputed as described in Section 6.1. Exposure will be calculated after the dates are imputed.

Extent of exposure (in days) will be compared between the treatment groups using the Wilcoxon Rank Sum test. In addition, the number of patients treated at each visit will be presented by treatment group.

Other summaries which will be presented are average dose (mg) per time period (3 months), ongoing status (yes, no, unknown) and primary reason for interruption/discontinuation (adverse drug reaction, lack of efficacy, disease improvement, patient/guardian decision, investigator decision, pregnancy and/or breast feeding).

All exposure parameters will be included in a by-patient listing.

15.6. Clinical Laboratory Tests

Analyses of clinical laboratory assessments will focus on serum creatinine, urine protein, urine albumin, and urine ACR. Other labs results will be provided in by-patient listings. Analysis will be done using the same methodology as for eGFR (Section 13.7).

Data will be summarized using descriptive statistics will be displayed for change from Analysis Start Date (time of diagnosis or treatment start) to e.g. time points such as the date of the first treatment switch, date of the treatment to migalastat or any other time points of interest.

Analysis Methods

Data will be listed. Listings will contain flags indicating therapy change. Spaghetti plots will indicate what treatment group a patient was on at the time of measurement.

In the Safety Population, data will be analyzed from the time of diagnosis.

For dialysis and transplant patients, the Analysis Start Date creatinine (as it is done for eGFR in Section 13.7) will be reset to the last value before dialysis start or before transplantation and used thereafter.

All laboratory data, values, units, normal reference range, and out-of-range flags collected in the clinical database will be included in by-patient listings for further medical review.

The analysis will address data based on the actual exposure. The count of out of range values as well as counts of clinically significant results will be presented by treatment.

Summaries of out of range values and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy. .

An additional table showing if a test result was out of range at any measurement over time will be prepared. A similar table will be prepared for potentially clinically significant values.

15.7. Vital Signs

Vital signs will be analyzed in the same way as clinical laboratory tests (Section 15.6).

Vital signs will also be collected at the time of a serious adverse event.

Those values will be included in a table that presents if a test result was out of range at any measurement. A similar table will be prepared for potentially clinically significant values.

15.8. Body Weight

Body weight will be analyzed in the same way as clinical laboratory tests (Section 15.6).

15.9. Electrocardiogram

Echocardiograms will be analyzed in the same way as clinical laboratory tests (Section 15.6).

15.10. Echocardiogram

Echocardiograms will be analyzed in the same way as clinical laboratory tests (Section 15.6). Echocardiogram parameters include LVMi, ejection fraction, fractional shortening, left ventricular internal diameter end diastole and end systole, midwall fractional shortening, and wall thickness. Echocardiograms will only be required if they are medically necessary.

15.11. Male Infertility

To show the occurrence of male infertility, a summary will be provided using binary variable (Yes/No) by treatment group and overall. Treatment group at enrollment will be utilized.

A listing will also be provided displaying the above information.

15.12. Pregnancy

A summary on the pregnancy status (Yes/No) by treatment group and overall will be provided. Treatment group at enrollment will be utilized.

A listing will also be provided displaying the above information.

15.13. Infusion Related Reactions

Infusion reactions will be reported during the ERT treatment.

16. EXPLORATORY ORGAN SPECIFIC ENDPOINTS

16.1. Lung Capacity

The following will be analyzed: clinically significant changes in spirometry measures of obstructive and restrictive lung function evaluated by body plethysmography:

- Forced expiratory volume after 1 second (FEV1)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

- Forced vital capacity (FVC)

  A clinically relevant change is defined as 25% decrease from baseline or as incident volume of 20% below predicted value according to an age- and sex-adjusted reference range.

Summaries of clinically relevant changes and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy. .

An additional table showing if a test result was clinically relevant at any measurement time point will be prepared.

16.2. Central and Peripheral Neurological Assessments

The assessments include:

- Standardized sweat test (hypo, normo, hyperhidrosis), standard unit of measure: mg pr. 30 minutes

Continuous data (mg per 30 min) will be analyzed in the same way as laboratory tests (Section 15.6).

Categorical test results (hypo, normo, hyperhidrosis) will be analyzed using counts. Shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy.

- Autonomic nerve function (small fibre assessment)
- Presence of Fabry associated abnormalities by brain MRI

Summaries of binary values (presence/absence) and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy.

An additional table showing a test result indicating presence of an abnormality anytime will be prepared.

16.3. Standardized Tilt-Test

- Presence of orthostatism (yes/no)
- Heart rate variability (Age-dependent – impaired vs not)

Summaries of binary values (presence/absence) and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy.

An additional table showing a test result indicating presence of an abnormality anytime will be prepared.

- Orthostatic blood pressure will be analyzed in the same way as laboratory tests (Section 15.6)

16.4. Audiological Assessments

- Audiogram (Impaired vs not)

Summaries of binary values (presence/absence) and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy.

An additional table showing a test result indicating presence of an abnormality anytime will be prepared.

16.5. Ophthalmological and Dermatological Assessments

The following will be analyzed:

- Presence of cornea versiculata
- Presence of angiokeratoma

Summaries of binary values (presence/absence) and shift tables (using Analysis Start Date as baseline) could be prepared for timepoints of interest such as e.g. start of migalastat therapy.

An additional table showing a test result indicating presence of an abnormality anytime will be prepared.

16.6. Cardiac Function

In addition to ECG and echocardiograms whose analysis is described previously, NT-proBNP and high sensitive Troponin T – will be analyzed from the Danish Fabry biobank using the same methodology as for clinical laboratory tests (Section 15.6).

16.7. Composite Phenotypic Score

Mainz Severity Score Index (Composite score) will be analyzed biobank using the same methodology as for clinical laboratory tests (Section 15.6).

16.8. Renal Function

Renal function analyses have been already described in the Effectiveness and Safety sections. An additional endpoint is a measured glomerular filtration rate by 99Tc-DTPA or 51Cr-EDTA. It will be analyzed in the same way as clinical laboratory tests (Section 15.6).

16.9. Patient Reported Outcomes

The PRO data will be analyzed in the Effectiveness Population. The PRO data will be summarized using descriptive statistics. The following PROs will be analyzed:

- SF-36 Health questionnaire (composite and Physical and mental components)
- NHP-Health Profile (Daily living questionnaire – composite score)
- Major Depression Inventory (Composite score)
- Composite score: Mainz Severity **Score** Index

The PRO measures will be scored using appropriate scoring algorithms specific for each questionnaire, which handle missing data for some of the questionnaire questions. Descriptive statistics will be displayed for change in PROs from time of diagnosis to each switch (or by time interval), by treatment group.

Statistical analysis will be performed using the same methodology as for the analysis of eGRF (Section 13.7).

17. PLANNED ANALYSES

17.1. Statistical Analysis Changes from the Protocol

None.

17.2. Interim Analyses

None

17.3. Final Analyses

Final analysis will be performed when all enrolled patients regardless how long they stayed in the registry.

18. REFERENCES

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19. SCHEDULE OF ASSESSMENTS

Not applicable.