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STATISTICAL ANALYSIS PLAN

A Multicenter, Multinational Study of the Effects of Fabrazyme® (agalsidase beta) Treatment on Lactation and Infants

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE:	Adverse Event
MCC:	Maternal Cell Contamination
NA:	Not Applicable
PK:	Pharmacokinetic

1 OVERVIEW AND INVESTIGATIONAL PLAN

1.1 STUDY DESIGN AND RANDOMIZATION

This is a phase 4, multicenter, multinational study of the potential effects of Fabrazyme on lactation and on the growth, development, and immunologic response of infants born to mothers with Fabry disease who are treated with Fabrazyme during lactation. The mothers will receive Fabrazyme treatment as part of the standard of care of Fabry disease and will be followed through the Fabry Registry.

Pregnant women who received Fabrazyme at any point during their pregnancy can be enrolled in the study. The mothers will receive Fabrazyme treatment (commercially available) at their currently prescribed dosage regimen as determined by their treating physician. If an enrolling woman is not receiving treatment with Fabrazyme at the Baseline visit, she should start treatment with Fabrazyme between the delivery and Month 1 visit.

Approximately 10 mothers and up to 10 infants will be enrolled in this study.

1.2 OBJECTIVES

1.2.1 Primary objectives

The primary objectives of this study are:

1. To determine whether αGAL activity is present in the breast milk of mothers with Fabry disease who are being treated with Fabrazyme during lactation.
2. To measure breast milk production and composition (volume, protein and fat content) in women with Fabry disease who receive Fabrazyme during lactation.
3. To determine whether Fabrazyme affects the growth, development, and immunologic response of infants born to mothers with Fabry disease who receive Fabrazyme during lactation.

1.2.2 Secondary objectives

NA

1.3 DETERMINATION OF SAMPLE SIZE

No formal statistical sample size calculations were performed for this study. The sample size of approximately 10 mothers and up to 10 infants is based upon 1) the availability of mothers to study lactation in this rare disease and 2) the availability of infants whose parents or legal guardians consent to their participation in this study.

1.4 STUDY PLAN

This study will evaluate the effects of Fabrazyme on lactation and on the growth, development, and immunologic response of infants born to mothers with Fabry disease who are treated with Fabrazyme during lactation. There are 3 participation scenarios: mother/infant full participation, mother full participation/infant development only, and mother full participation/infant no participation (see protocol Section 9.1).

Whether or not the mother continues to lactate will be assessed at each visit. Breast milk will be collected at Months 1, 3, and 6 immediately prior to Fabrazyme infusion, just after the end of the infusion, and 2 hours after the end of the infusion. Breast milk samples will be analyzed for volume, presence of αGAL activity, total fat content, and total protein concentration. Blood samples from the mother will be drawn for pharmacokinetic (PK) analysis prior to each breast milk collection. If infant genotyping is performed on the umbilical blood cord sample collected at Baseline visit, a buccal cell sample will be obtained from the mother to rule out contamination of the cord blood sample with maternal DNA.

If the parent(s)/legal guardian(s) consent to infant participation, the infant's growth and development will be assessed. The infant may also be tested for the formation or continued presence of serum IgG and IgM antibodies to r-hαGAL and for genotype.

For the purposes of this study, the mother and the infant will each be considered a study patient. The mother must be enrolled in the Fabry Registry and will be followed using the Fabry Registry assessment schedule and CRFs as well as this study's CRF. During this study, infants will be evaluated, ie, the infant may be tested for IgG and IgM antibodies to r-hαGAL at birth and Months 2, 6, and 12 and will be tested developmentally at each visit for 24 months, or until the parent(s)/legal guardian(s) withdraws consent and discontinues the infant's study participation, the infant is discontinued from the study by the Investigator (see Section 7.3.1), or the study is terminated (see Section 7.3.2).

If the mother is no longer lactating, the mother will discontinue this study but continue to be followed in the Fabry Registry. The infant will be followed for development only for the remainder of this 24-month study.

1.5 MODIFICATIONS TO THE STATISTICAL SECTION OF THE PROTOCOL

The statistical section of the protocol was never changed in an amendment.

The first patient was enrolled on August 29, 2006. After 5 mothers have contributed breast milk samples to this study, an interim analysis of αGAL activity in the breast milk will be performed. In addition, the status of this study will be summarized annually as part of the Fabry Registry Program Annual Report and the Fabrazyme Post-Marketing Commitment Annual Report..

1.6 STATISTICAL MODIFICATIONS MADE IN THE STATISTICAL ANALYSIS PLAN

NA

2 STATISTICAL AND ANALYTICAL PROCEDURES

2.1 ANALYSIS ENDPOINTS

2.1.1 Demographic and baseline characteristics

The baseline value is defined as within 1 month prior to delivery (mother) and at birth (infant).

All baseline safety parameters are presented along with the on-treatment summary statistics in the safety sections (Section 2.4.5 and Section 2.4.4).

Demographic characteristics

Demographic variables are gender (Male, Female), race (white, Black, Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Island), age in years (quantitative), ethnicity (Hispanic or Latino, non-Hispanic or Latino).

Lactation Status

The mother's lactation status will be provided: Is the mother providing milk for the protocol? (Yes, No); Is the mother planning on breast feeding the infant(s)? (Yes, No).

Maternal cell contamination (MCC) analysis

If umbilical cord blood sample was collected and MCC analysis was performed, the results (Maternal Cell Contamination detected, No Maternal Cell Contamination detected) will be collected.

Medical history

Medical history (including Fabrazyme status during pregnancy) will be collected through the Registry.

This information will be coded using the version of Medical Dictionary for Regulatory Activities (MedDRA) currently in effect at Sanofi at the time of database lock.

Any technical details related to computation, dates, and imputation for missing dates are described in Section 2.5.

2.1.2 Prior or concomitant medications

All medications taken from the time of signing the informed consent through study completion are to be reported in the case report form pages.

All medications will be coded using the World Health Organization-Drug Dictionary (WHO-DD) using the version currently in effect at Sanofi at the time of database lock.

Any technical details related to computation, dates, imputation for missing dates are described in Section 2.5.

2.1.3 Efficacy endpoints

NA

2.1.3.1 Primary efficacy endpoint(s)

NA

2.1.3.2 Secondary efficacy endpoint(s)

NA

2.1.4 Safety endpoints

The safety analysis will be based on the reported adverse events (AEs) and other safety information, such as physical examinations, etc.

The following study variables will be summarized as appropriate for the mothers (items marked with * will be collected through the Registry):

- Medical history (including Fabrazyme status during pregnancy)*
- Genotyping*
- Pregnancy outcome*
- IgG antibody status*
- Lactation status
- Plasma and breast milk PK testing
- The level of αGAL, volume, and fat and protein content in breast milk samples
- Frequency of AEs
- Frequency of concomitant medications

The following study variables will be summarized as appropriate for the infants:

- Medical history
- Physical examination
- Gender

- Genotype
- Apgar score at 1 minute and 5 minutes after birth
- Denver II Developmental Screening Test
- IgM antibodies to r-hαGAL – presence, time to development, level of titers present
- IgG antibodies to r-hαGAL – presence, time to development, level of titers present
- Frequency of AEs
- Frequency of concomitant medications

Observation period

The on-study observation period is defined as the time from the signing of the informed consent until the end of the study (defined as last protocol planned patient contact by telephone approximately 28 days after the final study procedures are completed).

2.1.4.1 Adverse events variables

All AEs ongoing at the time of withdrawal, study termination, or study completion require a follow up at approximately 28 days after discontinuation of study participation. The Investigator will be asked to follow-up on all SAEs that were ongoing at the time of withdrawal, termination or completion until resolution, until both the sponsor and investigator agree follow-up is deemed no longer medically necessary or until the patient is lost to follow-up.

If any new SAEs are identified during the course of this follow-up of ongoing SAEs, additional follow-up of these new SAEs may be performed. The Investigator and sponsor will determine if the follow-up is clinically warranted and relevant to the evaluation of the safety of the trial treatment or until patient is lost to follow up. The investigator must follow patients with AEs until their condition resolves or stabilizes. Certain conditions that are not expected to resolve, such as metastatic cancer, need not be followed indefinitely by the investigator.

All adverse events (including serious adverse events) will be coded to a lower-level term (LLT), preferred term (PT), high-level term (HLT), high-level group term (HLGT), and associated primary system organ class (SOC) using the version of Medical Dictionary for Regulatory Activities (MedDRA) currently in effect at Sanofi at the time of database lock.

All occurrences of adverse events (including serious adverse) from the time of signed informed consent until the end of the study will be recorded.

2.1.4.2 Deaths

The deaths from the signing of the informed consent through the completion of the study will be recorded.

2.1.4.3 Laboratory safety variables

NA

2.1.4.4 Vital signs variables

NA

2.1.4.5 Electrocardiogram variables

NA

2.1.5 Pharmacokinetic variables

Pharmacokinetic Sampling

αGAL concentrations will be measured form Blood and breast milk samples taken from the mothers. Blood samples will be taken from the mother at Months 1, 3, and 6 for PK testing (if mother is lactating at the time of the evaluation and is receiving Fabrazyme therapy).

Bioanalytical Method

Plasma and milk αGAL concentrations will be quantified by measuring enzyme activity using a fluorometric assay with a lower limit of quantification of 1.38 ng/mL. The assay does not differentiate between endogenous αGAL and Fabrazyme.

Pharmacokinetic parameters

The following pharmacokinetic parameters will be calculated, using noncompartmental methods from plasma αGAL concentrations and milk αGAL concentrations

- maximum plasma concentration observed (C_{max}) in plasma and milk
- area under the plasma αGAL concentration versus time curve calculated using the trapezoidal method from 0 to 2 hours post end of infusion ($AUC_{0\text{-}2\ plasma}$).
- the amount of αGAL in milk calculated as the product of αGAL activity times the milk volume
- area under the milk αGAL concentration versus time curve calculated using the trapezoidal method from 0 to 2 hours post end of infusion ($AUC_{0\text{-}2\ milk}$).

- lactation clearance determined in the 0-2 hours interval, according to the following equation: amount of αGAL excreted over the sampling period divided by the AUC during the sampling period

The milk to plasma ratio for AUC_{0-2} will also be computed.

2.2 DISPOSITION OF PATIENTS

This section describes patient disposition for both patient study status and the patient analysis populations.

Screened patients are defined as any patient who met the inclusion criteria and signed the informed consent. Enrolled patients are defined as any patient who signed the informed consent and participated in at least one study visit.

For patient study status, the total number of patients in each of the following categories will be presented in the clinical study report using a flowchart diagram or summary table:

- Screened patients
- Screen failure patients and reasons for screen failure
- Patients who did not complete the study treatment period as per protocol
- Patients who discontinued study treatment by main reason for permanent treatment discontinuation
- Status at last study contact

For all categories of patients (except for the screened and nonrandomized categories) percentages will be calculated using the number of enrolled patients as the denominator. Reasons for treatment discontinuation will be supplied in tables giving numbers and percentages by treatment group.

A patient is considered lost to follow-up at the end of the study if he/she is not assessed at the last protocol planned visit.

2.2.1 Randomization and drug dispensing irregularities

NA

2.3 ANALYSIS POPULATIONS

All patients enrolled will be included in the safety population. Patients who signed the informed consent but did not participate in any study visit are not considered enrolled in the study.

2.3.1 Efficacy populations

NA

2.3.2 Safety population

The safety population is defined as all patients who enrolled, i.e. signed the informed consent and participated in at least 1 study visit.

2.4 STATISTICAL METHODS

The descriptions of analysis below assume that a sufficient number of patients will be enrolled in the study such that summary statistics of the data will be meaningful to the reviewers. In case of sparse data (less than 5 mothers and/or 5 infants enrolled), the collected data will be presented in patient listings.

2.4.1 Demographics and baseline characteristics

Continuous data will be summarized using the number of available data, mean, standard deviation (SD), median, minimum, and maximum for each treatment group. Categorical and ordinal data will be summarized using the number and percentage of patients in each treatment group.

Parameters described in Section 2.1.1 will be summarized by mother and infant groups using descriptive statistics.

Medical history as collected in the Registry will be presented according to the preprinted case report form history responses.

2.4.2 Prior or concomitant medications

The concomitant medications will be presented for the enrolled population.

Medications will be summarized by treatment group according to the WHO-DD dictionary, considering the first digit of the anatomic category (ATC) class (anatomic category) and the first 3 digits of the ATC class (therapeutic category). All ATC codes corresponding to a medication will be summarized, and patients will be counted once in each ATC category (anatomic or therapeutic) linked to the medication. Consequently, patients may be counted several time for the same medication.

The table for prior medications will be sorted by decreasing frequency of ATC followed by all other therapeutic classes based on the overall incidence across treatment groups. In case of equal frequency regarding ATCs, alphabetical order will be used.

The tables for concomitant medications will be sorted by decreasing frequency of ATC followed by all other therapeutic classes based on the incidence. In case of equal frequency regarding ATCs, alphabetical order will be used.

2.4.3 Extent of investigational medicinal product exposure and compliance

The enrolled mothers will receive Fabrazyme treatment (commercially available) at their currently prescribed dosage regimen as determined by their treating physician. Therefore, the extent of IMP exposure and compliance will not be assessed nor summarized.

2.4.3.1 Extent of investigational medicinal product exposure

The data collected for Fabrazyme infusions received by the mother will be presented in a listing.

2.4.3.2 Compliance

NA

2.4.4 Analyses of efficacy endpoints

2.4.4.1 Analysis of primary efficacy endpoint(s)

NA

2.4.4.2 Analyses of secondary efficacy endpoints

NA

2.4.4.3 Multiplicity issues

NA

2.4.4.4 Additional efficacy analysis(es)

NA

2.4.5 Analyses of safety data

The summary of safety results will be presented by mother and infant groups.

General common rules

All safety analyses will be performed on the safety population as defined in Section 2.3.2. In case of sparse data, the results will be presented in patient listings.

2.4.5.1 Analyses of adverse events

Generalities

All adverse events (AEs) reported from signing of informed consent through completion of the study will be presented.

Adverse event incidence tables will present by SOC and PT, sorted in alphabetical order for each group (mother, infant), the number (n) and percentage (%) of patients experiencing an adverse event. Multiple occurrences of the same event in the same patient will be counted only once in the tables within the study period. The denominator for computation of percentages is the safety population within each group (mother, infant).

Sorting within tables ensures the same presentation for the set of all adverse events within the observation period. For that purpose, the table of all adverse events presented by SOC and PT sorted by the internationally agreed SOC order and decreasing frequency of PTs within SOCs will define the presentation order for all other tables unless otherwise specified.

Analysis of all adverse events

The following adverse event summaries will be generated for the safety population.

- Overview of adverse events, summarizing number (%) of patients with any
  - adverse event
  - serious adverse event
  - adverse event leading to death
  - adverse event leading to permanent treatment discontinuation
- All adverse events by primary SOC and PT, showing the number (%) of patients with at least 1 adverse event, sorted by the internationally agreed SOC order and by decreasing incidence of PTs within each SOC. This sorting order will be applied to all other tables, unless otherwise specified
- All adverse events regardless of relationship and related to IMP, by primary SOC and PT, showing the number (%) of patients with at least 1 adverse event
- All adverse events by maximal severity, presented by primary SOC and PT, showing the number (%) of patients with at least 1 adverse event by severity (ie, mild, moderate, or severe)

Analysis of all treatment emergent serious adverse event(s)

- All serious adverse events by primary SOC and PT, showing the number (%) of patients with at least 1 serious adverse event
- All serious adverse events regardless of relationship and related to IMP, by primary SOC and PT, showing the number (%) of patients with at least 1 serious adverse event

- All serious adverse events by maximal severity, presented by primary SOC and PT, showing the number (%) of patients with at least 1 serious adverse event by severity (ie, mild, moderate, or severe)

Analysis of all adverse event(s) leading to treatment discontinuation

- A listing of all adverse events leading to treatment discontinuation will be presented

2.4.5.2 Deaths

A listing of adverse events leading to death (death as an outcome on the adverse event case report form page as reported by the Investigator) will be presented.

2.4.5.3 Analyses of laboratory variables

NA

2.4.5.4 Analyses of vital sign variables

NA

2.4.5.5 Analyses of electrocardiogram variables

NA

2.4.5.6 Analyses of other safety endpoints

2.4.6 Analyses of pharmacokinetic and pharmacodynamic variables

Descriptive statistics of pharmacokinetic (PK) parameters by visit and by mother and infant groups will be provided. However, in case of sparse data, a by patient listing will be provided.

2.5 DATA HANDLING CONVENTIONS

2.5.1 General conventions

None.

2.5.2 Data handling conventions for secondary efficacy variables

NA

2.5.3 Missing data

For categorical variables, patients with missing data are not included in calculations of

percentages unless otherwise specified. When relevant, the number of patients with missing data is presented.

Handling of medication missing/partial dates

No imputation of medication start/end dates or times will be performed. If a medication date or time is missing or partially missing and it cannot be determined whether it was taken prior or concomitantly, it will be considered a concomitant medication.

Handling of adverse events with missing or partial date/time of onset

Missing or partial adverse event onset dates and times will not be imputed. No imputation of adverse event end dates/times will be performed. No imputation is planned for date/time of adverse event resolution.

Handling of missing assessment of relationship of adverse events to investigational medicinal product

If the assessment of the relationship to IMP is missing, then the relationship to IMP has to be assumed and the adverse event considered as such in the frequency tables of possibly related adverse events, but no imputation should be done at the data level. No imputation of relationship will be presented in the listings.

Handling of missing severity of adverse events

If the severity is missing for 1 of the occurrences of an adverse event, the maximal severity on the remaining occurrences will be considered. If the severity is missing for all the occurrences, a "missing" category will be added in the summary table.

2.5.4 Windows for time points

None

2.5.5 Unscheduled visits

Unscheduled visit measurements will not be presented in by-visit summaries but will be presented in listings.

2.5.6 Pooling of centers for statistical analyses

All analyses will be performed on the safety population.

2.5.7 Statistical technical issues

None

3 INTERIM ANALYSIS

After 5 mothers have contributed breast milk samples to this study, an interim analysis of αGAL activity in the breast milk will be performed.

In addition, the status of this study will be summarized annually as part of the Fabry Registry Program Annual Report and the Fabrazyme Post-Marketing Commitment Annual Report.

4 DATABASE LOCK

The database is planned to be locked at 60 days after last patient last visit.

5 SOFTWARE DOCUMENTATION

All summaries and statistical analyses will be generated using SAS version 9.4 or higher.

6 REFERENCES

None

7 LIST OF APPENDICES

None

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