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Statistical Analysis Plan

Protocol number: NI-0501-07

Title: An observational, multicenter study to evaluate interferon gamma (IFNγ) and other inflammatory mediators in patients with malignancy-associated hemophagocytic lymphohistiocytosis (M-HLH)

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1 Abbreviations and definition of terms

AE	Adverse event
CSR	Clinical Study Report
FAS	Full Analysis Set
IFNγ	Interferon gamma
ICF	Informed consent form
LLOQ	Lower limit of quantification
HLH	Hemophagocytic lymphohistiocytosis
M-HLH	Malignancy-associated hemophagocytic lymphohistiocytosis
NI-0501	Emapalumab
pHLH	Primary hemophagocytic lymphohistiocytosis
SAE	Serious adverse event
SAP	Statistical Analysis Plan
Sobi	Swedish Orphan Biovitrum
ULOQ	Upper limit of quantification
PPS	Per Protocol Set

2 Introduction

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for Sobi protocol NI-0501-07 (An observational, multicenter study to evaluate interferon gamma (IFNγ) and other inflammatory mediators in patients with malignancy-associated hemophagocytic lymphohistiocytosis (M-HLH)).

This observational study is designed to provide a better understanding of the potential use of IFNγ and IFNγ-inducible chemokines as markers of M-HLH disease activity and potential prognosis and of the possible role of IFNγ as a therapeutic target in M-HLH, and to explore the genetic/ profiles in patients with M-HLH with regard to HLH-related genes in patients who are diagnosed with M-HLH.

The purpose of this SAP is to outline the exploratory analyses to be completed to support the synopsis for protocol NI-0501-07. The exploratory analyses identified in this SAP could be included in regulatory submissions and/or future manuscripts. Also, additional exploratory analyses not necessarily identified in this SAP may be performed to support the clinical

development program. Any post-hoc analyses not identified in this SAP will be clearly identified as such in the synopsis.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the FDA and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Guidance on Statistical Principles in Clinical Trials. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association and the Royal Statistical Society, for statistical practice.

3 Study objectives and endpoints

3.1 Study objectives

- To determine the levels of inflammatory markers including, but not limited to interferon gamma (IFNγ), interleukin 1 beta (IL1β), interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 17 (IL-17), soluble IL-2 Receptor α (sCD25), C-X-C chemokine ligand 9 (CXCL9), C-X-C chemokine ligand 10 (CXCL10), C-XC chemokine ligand 11 (CXCL11), soluble CD163 (sCD163), neopterin and Tumor Necrosis Factor alpha (TNFα) in patients diagnosed with M-HLH and whenever possible, to monitor the evolution over time of these markers.
- To assess the relationship between the above-mentioned inflammatory cytokines and disease activity.
- To assess the relationship between genetic variants of the genes typically causing primary HLH and M-HLH and associated inflammatory markers.

3.2 Study endpoints

3.2.1 Inflammatory biomarkers

Level of

- CXCL10 (pg/mL)
- CXCL9 (pg/mL)
- IFNg V-PLEX (pg/mL)
- IL10 V-PLEX (pg/mL)
- IL1B V-PLEX (pg/mL)
- IL6 V-PLEX (pg/mL)
- Neopterin (nM)
- TNFa V-PLEX (pg/mL)
- Total hIFNγ (pg/mL)
- sCD163 (ng/mL)

- sIL2Rα (pg/mL), also called sCD25

Note: No data reported for C-XC chemokine ligand 11 (CXCL11) and interleukin 17 (IL-17).

The biomarkers are measured in the M-HLH group and in the malignant control group without M-HLH.

3.2.2 Inflammatory biomarkers- correlation

The degree of correlations between inflammatory markers and between selected inflammatory markers and markers of disease activity (lab assessments) will be investigated.

The inflammatory biomarkers are as listed in section 3.2.1 and the other laboratory assessments as defined in the protocol are listed below

- Albumin
- AST, ALT, Alkaline Phosphatase
- Basophils
- ß2-microglobulin
- Total and conjugated Bilirubin
- BUN, serum creatinine
- CRP
- D-dimers
- Hemoglobin, Hematocrit, Red blood cells, white blood cells and differential count, platelets
- Fasting triglycerides
- Ferritin
- Fibrinogen
- Lactate dehydrogenase
- NK cell activity
- Serum IgG
- Sodium

3.2.3 Genetic variants

Genetic data was only available for 5 out of 19 patients in the HLH group and no analysis of genetic variants will be performed.

4 Study methods

4.1 Overall study design and plan

This is a non-interventional study designed to determine the levels of pro-inflammatory markers (as listed above) in patients diagnosed with M-HLH and to assess the relationship between the biomarkers and disease activity and prognosis during the M-HLH course. Inflammatory markers will also be measured in a group of control patients diagnosed with hematological malignancy in absence of HLH (as described above).

Data collection may be performed retrospectively if sufficient clinical information is available to allow for a meaningful interpretation of the biomarker results.

An approximate volume of 500 µl – 1 ml of serum or plasma is required per time point. Whenever possible, serum (and not plasma) should be collected, as this is the preferred matrix for the biomarker analysis.

In addition to the serum samples for the biomarker analysis, relevant information gathered by the treating physician will be collected in a data collection form. This will include information on the clinical presentation of M-HLH, the type of underlying malignancy, the date of onset of malignancy in relation to HLH manifestations, treatment regimen prior to and/or ongoing at the time of M-HLH onset, laboratory parameters, M-HLH disease activity (e.g. newly diagnosed, reactivation), specific HLH therapy with best response to the therapy, other concomitant medications, patient follow-up and disposition, stem cell transplant status, duration of response, and survival. Whenever possible, collection of serum samples for biomarker analysis and relevant information should occur at M-HLH diagnosis, at regular time intervals during the treatment course, at resolution and/or reactivation of the disease.

In addition to the above a minimum of 1 ml of whole blood will be collected (EDTA tubes) for genetic characterization and identification of the presence of genetic variants of the genes typically causing primary HLH. A specific consent for genetic testing must be given by the patient. Genetic samples may also be collected from existing bank of samples (frozen whole blood in EDTA or frozen extracted DNA), if proper consent has been obtained.

4.2 Selection of study population

Male and female (adult and children) patients who are diagnosed with M-HLH and that meet the inclusion criteria. In addition, patients with a diagnosis of hematological malignancy in the absence of HLH will be included as a control group.

4.3 Method of treatment assignment and randomization

This is an observational study (not randomized). Please see details on study population section 4.2

5 Sequence of planned analysis

5.1 Interim analyses

There was no planned interim analysis for this observational study.

5.2 Analyses and reporting

All final analyses identified in the protocol and in this SAP will be performed only after the database lock. A data review will be held prior to database lock. The SAP will be finalized, locked and signed prior to database lock.

Any post-hoc analyses included in the study synopsis which were not identified in this SAP, will be clearly identified as such in the relevant report section.

6 Sample size determination

No formal sample size was calculated for this observational study.

The protocol stated a minimum of 14 (maximum of 50) M-HLH patients will be studied, including a minimum of 7 non iatrogenic M-HLH and 7 iatrogenic M-HLH. A control group of 10 patients with a diagnosis of hematological malignancy in absence of HLH will also be evaluated.

A total of 39 patients where included in the study, 19 patients were diagnosed with M-HLH and 20 patients were included in the control group.

7 Analysis populations

The All enrolled set will be used in the statistical analyses as no study drug was administered.

7.1 All enrolled set

All subjects who signed an informed consent.

8 General issues for statistical analysis

No statistical tests will be presented. Only exploratory descriptive statistics will be displayed as described below:

Continuous data will be summarized using descriptive statistics: n, mean, standard deviation (SD), median, minimum, 1st quartile, 3rd quartile and maximum, unless otherwise indicated.

Minimum and maximum will be presented to the same number of decimal places as the raw data and mean, standard deviation and median will be presented to one more decimal place than the raw data.

Categorical data will be summarized using counts and percentages. Percentages will be suppressed when the count is zero, however the category will still be displayed. The denominator for all percentages will be the number of patients within the group for the population of interest, unless otherwise indicated. Percentages will be presented to one decimal place.

All data will be listed in individual patient data listings.

Statistical analyses will be performed using SAS software Version 9.4 or later (SAS Institute Inc, Cary, North Carolina, United States).

8.1 Handling of missing data and outliers

No imputation of missing data will be performed.

8.2 Multicenter studies

Not applicable. This observational study was conducted in one site.

8.3 Multiple comparisons and multiplicity

No comparison and multiplicity adjustment will be performed, only descriptive statistics.

8.4 Derived and computed variables

8.4.1 Age

Age at informed consent (years) will be calculated as:

$$Age\ (years) = \frac{Date\ of\ informed\ consent - \ Date\ of\ birth}{365.25}$$

Age at diagnosis of malignancy (years) will be calculated as:

$$Age \text{ at diag. of malignancy } (years) = \frac{\text{Date of diagnosis of malignancy} - Date\ of\ birth}{365.25}$$

Age at HLH diagnosis (years) will be calculated as:

$$Age\ at\ HLH\ diag.\ (years) = \frac{\text{Date of HLH diagnosis} - \ Date\ of\ birth}{365.25}$$

Ages will be rounded to the closest whole year for presentation in the summary tables and listings.

8.4.2 LLOQ and ULOQ

Values reported as below the lower limit of quantification (LLOQ) will be replaced by LLOQ/2 and values above upper limit of quantification (ULOQ) will be replaced by ULOQ prior to the statistical analyses.

9 Patient disposition

Not applicable, all patients who signed an ICF will be enrolled in the study. No study treatment is given.

10 Demographics and baseline characteristics

10.1 Demographics

Demographic characteristics will be summarized by HLH patients, non-HLH patients and overall for the All enrolled set:

- Age (years) at ICF
- Sex: Male, Female
- Weight (kg) at ICF
- Height (cm) at ICF

Demographic data will also be listed.

10.2 Medical History

Medical History characteristics (malignancy and HLH) will be summarized by HLH patients, non-HLH patients and overall, for the All enrolled set and will include:

- Malignancy
  - Age at diagnosis of malignancy (years)
  - Type of malignancy
    - Type of malignancy will be listed as entered in the CRF and summarized as coded.
- HLH (only applicable to the HLH patients)
  - Age at HLH diagnosis (years)
  - Chemotherapy induced malignancy: Yes, No, Not Tested
  - Molecular diagnosis relevant for HLH: Yes, No, Not Tested

- Mutation (if molecular diagnosis): Homozygous, Heterozygous
- Mutation (if molecular diagnosis): PRF1, UNC13D, STX11, RAB27A, STXBP2, SH2D1A, LYST
- Infectious trigger detected: Yes, No, Not Tested
  Note: type and subtype if available, e.g. Virus (EBV, CMV,…), fungi, bacteria will be listed.
- HLH 2004 criteria that were met
  - Fever: Yes, No
  - Splenomegaly: Yes, No
  - Hb <90 g/L: Yes, No, Not Applicable
  - Platelets $<100*10^9$/L: Yes, No, Not Applicable
  - Neutrophils $<1*10^9$/L: Yes, No, Not Applicable
  - Hypofibrinogenaemia: Yes, No, Not Tested
  - Hypertriglyceridaemia: Yes, No, Not Tested
  - Increased level of sCD25: Yes, No, Not Tested
  - Evidence of haemophagocytosis: Yes, No, Not Tested
  - Decreased or absent NK cell cytotoxicity: Yes, No, Not Tested
  - Additional supportive evidence: CNS involvement, Hepatomegaly

Medical History Characteristics data will also be listed.

No medical history apart from HLH and malignancy is collected.

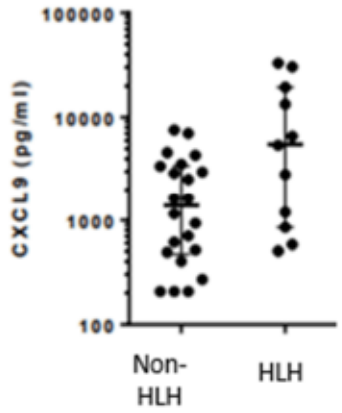
11 Prior and concomitant medication

Prior and concomitant medications are collected as free text and will not be coded and summarized, only listed as entered in the CRF.

12 Endpoint analyses

12.1 Inflammatory biomarkers

The inflammatory biomarkers as listed in section 3.2.1 will be displayed in scatter plots grouped by HLH and non-HLH group as in the below example.



For the HLH group the biomarker value closest to the date of HLH diagnosis (value must be after HLH diagnosis) will be used if more than one sample is available. For the non-HLH group the minimum and maximum value will be used and displayed in different colours/markers. If just one value available that value will be displayed with the same colour/marker as the HLH group.

Biomarker data will be summarized by group using descriptive statistics and presented for HLH patients, non-HLH patients and overall.

12.2 Inflammatory biomarkers - correlations

The degree of correlations between inflammatory markers and between inflammatory markers and laboratory assessments (potential markers of disease activity) will be assessed.

The correlations to be performed are:

1. All inflammatory biomarkers vs. all other inflammatory biomarkers as listed in section 3.2.1
2. Biomarkers relevant for emapalumab (i.e. CXCL9, CXCL10, IFNγ and sIL2Rα) vs. all laboratory assessments as listed in section 3.2.2

The correlations will be evaluated by plotting biomarker scores i.e., CXCL9 vs. CXCL10 (for all combinations) in scatter plots. In the scatter plots the groups (HLH and non-HLH) will be represented by different colors. The biomarker value closest to the date of HLH diagnosis will be used if more than one sample available. For the non-HLH group the minimum and maximum value will be displayed with different markers. If just one value available that value will be displayed with another marker.

Data will be investigated to confirm normal or log-normal distribution of the parameters. If normal, the actual values will be plotted. If not normal, log-tranformed values will be plotted.

The correlated samples should be within a time frame of three days.

12.3 Genetic variations

Not enough data available.

12.4 Subgroup Analyses

Data will be stratified and presented separately for HLH patients and non-HLH patients (i.e. control group). No additional subgroup analysis will be performed.

13 Safety analyses

Not applicable, study is observational.

13.1 Adverse events

Not Applicable as adverse events are not collected.

13.2 Laboratory data

A subset of the laboratory data collected will be displayed in scatter plots the same way as the biomarker data described in section 12.1 grouped by HLH and non-HLH group. The lab data to be plotted are:

- AST, ALT, Alkaline Phosphatase
- Total and conjugated Bilirubin
- CRP
- D-dimers
- white blood cells and differential count, platelets
- Ferritin
- Fibrinogen
- Lactate dehydrogenase

For the HLH group the value closest to the date of HLH diagnosis (value must be after HLH diagnosis) will be used if more than one sample is available. For the non-HLH group the minimum and maximum value will be displayed in different colours/markers. If just one value available that value will be displayed with the same colour/marker as the HLH group.

Laboratory data will be summarized by group using descriptive statistics and presented for HLH patients, non-HLH patients and overall.

All laboratory data will be listed by patient.

13.3 Vital signs

Weight and Height are only collected at screening/ICF. They will presented as part of the Demographic table (see Section 10.1).

14 Changes from the planned analyses in the clinical trial protocol

No data reported for C-XC chemokine ligand 11 (CXCL11) and interleukin 17 (IL-17), hence no analyses performed for these biomarkers.

Genetic information was only obtained from 5 out of 19 HLH patients, no analysis on genetic variants will be performed.

15 References

N/A