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Statistical Analysis Plan

Protocol number:	NI-0501-08
Title:	An observational, multicenter study to evaluate levels of Interferon gamma (IFNγ) and other inflammatory mediators in Adult Patients with Hemophagocytic Lymphohistiocytosis (A-HLH)
SAP Author:	PPD
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1 Abbreviations and definition of terms

AE	Adverse event
CSR	Clinical study report
DCT	Data collection tool
FAS	Full analysis set
IFNγ	Interferon gamma
ICF	Informed consent form
LLOQ	Lower limit of quantification
HLH	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-0501-08 (An observational, multicenter study to evaluate levels of Interferon gamma (IFNγ) and other inflammatory mediators in Adult Patients with Hemophagocytic Lymphohistiocytosis (A-HLH)).

The present observational study is designed to provide a better understanding of IFNγ and IFNγ inducible chemokines as markers of HLH disease activity in adults, and of the potential of IFNγ to represent a therapeutic target in this patient population.

Adult patients (≥18 years old) are eligible, if having consented to the use of their clinical data for research purposes. Clinical data and sample collection may be performed either retrospectively (provided that sufficient clinical information is available to allow for a meaningful interpretation of the biomarker results) and prospectively.

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If retrospective cases are evaluated, the presence of a malignancy and the diagnosis of primary HLH (defined as homozygous mutation in a known HLH causative gene) represent exclusion criteria for the study. On the contrary, if prospective patients are considered, inclusion in the study may occur before the diagnostic work-up has been completed. In case the diagnosis of malignancy-related HLH or primary HLH (as above defined) is made, the data collected will be analyzed separately.

The purpose of this SAP is to outline the exploratory analyses to be completed to support the synopsis for protocol NI-0501-08. Any post-hoc analyses not identified in this SAP will be clearly identified as such in the synopsis. 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.

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), interleukin 18 (IL-18), 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 adult patients diagnosed with A-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 explore the presence of genetic variants of the genes typically causing primary HLH in these patients and any association with the course of the HLH disease.

3.2 Study endpoints

3.2.1 Inflammatory biomarkers

Level of

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- 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), interleukin 17 (IL-17) and interleukin 18 (IL-18).

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
- PT and aPTT
- Serum IgG
- Sodium

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3.2.3 Genetic variants

Genetic data was obtained for 8 out of 14 patients, of these 4 had no known HLH mutation and the other 4 had mutations but none of them where diagnosed with pHLH. 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 adult patients diagnosed with HLH and to assess the relationship between the biomarkers and disease activity. Whenever possible, the evolution of inflammatory markers will be followed during HLH treatment and the relationship with the disease outcome explored.

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

Relevant clinical information gathered by the treating physician will be collected in a data collection form. This will include information on the clinical presentation of HLH, the assessments performed to characterize the secondary form, in particular those for identification of the potential HLH trigger, the date of onset of the background disease (if any) in relation to HLH manifestations, treatment regimen prior to and/or ongoing at the time of HLH onset, clinical and laboratory parameters relevant to HLH, 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.

As appropriate, collection of serum samples for biomarker analysis and relevant information should occur at HLH diagnosis, and at regular time intervals during the treatment course (not more than once a week) up to resolution of HLH. If patients present CNS involvement and lumbar puncture is being performed for diagnostic or therapeutic purposes, a CSF sample will be collected, if possible, for biomarker assessment, in particular CXCL9 and CXCL10.

If feasible and consistent with the standard clinical practice at the site, genetic characterization will be performed. 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 the patient's consent has been previously obtained.

Medical history, in particular all available information on search conducted for the identification of the potential HLH trigger needs to be collected.

4.2 Selection of study population

Male and female adult (≥18 years old) patients who are diagnosed with HLH and meet the inclusion criteria.

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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 a minimum of 14 (maximum of 40) adult patients with HLH will be studied, including a minimum of 7 patients with HLH of unknown origin (i.e. in whom a trigger for HLH cannot be identified).

A total of 14 patients where included in the study, 6 of whom had an unknown HLH trigger. Four patients diagnosed with a malignancy where included in the study.

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.

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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.

Four patients diagnosed with a malignancy (and hence classified as M-HLH) where included in the study, according to the protocol these patients are to be analysed separately. All data presentations will be split by M-HLH and A-HLH (all patients without malignancies).

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:

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

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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 for the All enrolled set and split by A-HLH and M-HLH groups:

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

Demographic data will also be listed.

10.2 Medical History

Medical History characteristics will be summarized for the All enrolled set and split by A-HLH and M-HLH groups and will include:

- HLH Diagnosis
  - Age at HLH diagnosis (years)
  - Molecular diagnosis relevant for HLH: Yes, No, Not Tested
  - Mutation (if molecular diagnosis): List as entered

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- CNS involvement
  - Bone marrow biopsy
    - Evidence of hemophagocytosis
      - List additional info if available
  - Other biopsy
    - Evidence of hemophagocytosis
      - List additional info if available
- Known underlying immunodeficiency
- Has HLH been diagnosed in any family member
- Functional tests at diagnosis
  - Decreased or absent NK cell cytotoxicity
  - Degranulation assay
  - Perforin expression
  - List comments if available
- HLH trigger detected: Yes, No, Not Tested
  - Age at diagnosis of triggering disease
    - Infection Yes/No
      - Type,pathogen and viral load will be listed if available
    - Rheumatologic disease
      - Type listed if available
    - Malignancy
      - Type and induction listed if available
    - Other HLH trigger
      - Details listed if available
- Other relevant diagnosis at sample procurement
- HLH criteria fulfilled
  - Fever
  - Splenomegaly
  - Cytopenias
  - Hypertriglyceridemia and/or hypofibrinogenemia
  - Hemophagocytosis in bone marrow
  - Ferritin (>500 ug/L)
  - sIL-2R (>2,400 U/mL)
  - Low or absent NK cell activity

Medical History Characteristics data will also be listed.

11 HLH outcome

HLH outcome will be summarized for the All enrolled set and split by A-HLH and M-HLH groups and will include:

- HLH resolved (Yes/no)

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- HLH reactivated (Yes/no)
- Has HSCT been performed (Yes/no)
- Is patient alive (Yes/no)

12 Prior and concomitant medication

12.1 HLH theraphy

- Treatments received (Steroids, etoposide,IVIg, CaNeur Inhibitors, Other)

List name of drug, dose frequency etc if available. Entered as free text in CRF and will not be coded.

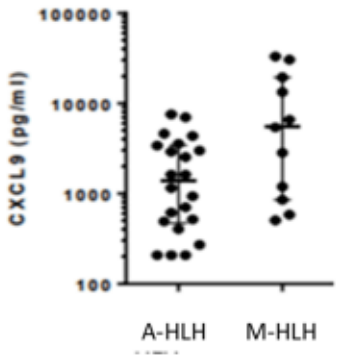
12.2 Concomitant medications (other than for HLH)

Concomitant medication at the time of sample procurement are collected as free text and will not be coded and summarized, only listed as entered in the DCT (Data Collection Tool).

13 Endpoint analyses

13.1 Inflammatory biomarkers

The inflammatory biomarkers as listed in section 3.2.1 will be displayed in scatter plots and split by A-HLH and M-HLH groups as in the below example.



If several samples available the biomarker value closest to the date of HLH diagnosis (value must be after HLH diagnosis) will be used.

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Biomarker data will be summarized for all patients by A-HLH and M-HLH groups using descriptive statistics.

13.2 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. The biomarker value closest to the date of HLH diagnosis will be used if more than one sample available. A-HLH and M-HLH patients will be shown in the plots with different colours or symbols.

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.

13.3 Genetic variations

Not enough data available.

13.4 Subgroup Analyses

Patients diagnosed as A-HLH and M-HLH will be analysed separately.

14 Safety analyses

Not applicable, study is observational.

14.1 Adverse events

Not Applicable as adverse events are not collected.

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14.2 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 13.1. 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

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.

Laboratory data will be summarized by A-HLH and M-HLH groups using descriptive statistics.

All laboratory data will be listed by patient.

14.3 Vital signs

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

15 Changes from the planned analyses in the clinical trial protocol

No data reported for C-XC chemokine ligand 11 (CXCL11) , interleukin 17 (IL-17) and interleukin 18 (IL-18), hence no analyses performed for these biomarkers.

No analysis on genetic variants will be performed.

16 References

N/A

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