STATISTICAL ANALYSIS PLAN

Protocol NI-0501-04

A Phase 2/3 Open-label, Single Arm, Multicenter Study to Assess Safety, Tolerability, Pharmacokinetics and Efficacy of Intravenous Multiple Administrations of NI-0501, an Anti-interferon Gamma (Anti-IFNγ) Monoclonal Antibody, in Pediatric Patients with Primary Hemophagocytic Lymphohistiocytosis (HLH)

Protocol Number: **(Version Date)**	NI-0501-04 United States US-P-IND#111015 Version 5.1, March 24, 2016 Europe EudraCT#2012-003632-23 Version 6.0, February 26, 2016	NI-0501-04 NCT number: NCT01818492
Name of Test Drug:	Emapalumab (NI-0501)	
Phase:	2/3	
Methodology:	Open label, Single arm, Multicenter study	

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SIGNATURE PAGE

Protocol Title:	A Phase 2/3 Open-label, Single Arm, Multicenter Study to Assess Safety, Tolerability, Pharmacokinetics and Efficacy of Intravenous Multiple Administrations of NI-0501, an Anti-interferon Gamma (Anti-IFNγ) Monoclonal Antibody, in Pediatric Patients with Primary Hemophagocytic Lymphohistiocytosis (HLH)
Sponsor:	Novimmune SA 14 Chemin des Aulx - 1228 Plan les Ouates Switzerland
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[redacted]

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidance's and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report (CSR).

[redacted]

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ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-drug antibodies
AE	Adverse events
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
aPTT	Activated Prothrombin Time
AST	Aspartate aminotransferase
ATG	Anti-thymocyte globulin
BSA	Body Surface Area
CBC	Complete blood cell
CNS	Central nervous system
CSF	Cerebrospinal fluid
CSR	Clinical study report
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EMA	European Medicines Agency
EOT	End of treatment
EOT 04	End of treatment in NI-0501-04 study
EOT 04/05	End of treatment overall (i.e., including treatment continuation in NI-0501-05 study)
EU	Europe
FDA	Food and Drug Administration
HIV	Human immunodeficiency virus
HLH	Haemophagocytic lymphohistiocytosis
HSCT	Hematopoietic Stem Cell Transplantation
ICH	International Conference on Harmonisation
IFNγ	interferon gamma
IRR	Infusion-related reaction
IV	Intravenous
IVIG	Intravenous immunoglobulin
LDH	Lactate dehydrogenase
mAb	Monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
PD	Pharmacodynamic
pHLH	Primary HLH
PK	Pharmacokinetic
PT	Prothrombin Time
SAP	Statistical analysis plan
SAE	Serious Adverse Event

Abbreviation	Definition
sCD25	soluble CD25 (ie, soluble IL-2 receptor)
SDx	Study day x
SSC	Scientific steering committee
TEAE	Treatment-emergent adverse event
US	United States
WHO	World Health Organization

1. INTRODUCTION AND OBJECTIVES OF ANALYSIS

1.1. Introduction

This document describes the plan for final statistical analysis and reporting of study NI-0501-04, which is an open-label, single arm, multicenter phase 2/3 study to assess safety, tolerability, pharmacokinetics (PK), and efficacy of intravenous (IV) multiple administrations of NI-0501, an anti-interferon gamma (anti-IFNγ) monoclonal antibody (mAb), in paediatric patients with primary haemophagocytic lymphohistiocytosis (HLH).

The NI-0501-04 study is performed both in the United States and in Europe according to twin protocols coded, respectively, NI-0501-04-US-P-IND#111015 and NI-0501-04-EudraCT#2012-003632-23 (see protocol synopsis in Section 2.1).

The NI-0501-04 study was initially designed and conducted as a pilot Phase 2 study, with the target of enrolling 10 evaluable patients with primary HLH (pHLH).

The original protocol foresaw enrolment of pHLH patients in whom the disease had re-activated after an initial response to conventional induction therapy.

Two amendments to the protocol were implemented to broaden the study population and allow the inclusion of:

i) pHLH patients who, having received conventional HLH therapy, showed no response or worsening, or were intolerant to conventional therapy (US protocol version 3.0, April 16 2014; EU protocol version 4.0, May 07 2014)

ii) treatment-naïve patients (US protocol version 4.0, November 17 2014; EU protocol version 5.0, December 15 2014)

Lastly, the protocol has been amended to continue as a Phase 2/3 study with enrolment of the same patient population, ie, pHLH patients who receive NI-0501 either as first line or as second line HLH treatment.

The principal objective of the study is to demonstrate the efficacy and safety of NI-0501 as second line treatment of pHLH, and in this respect the pivotal cohort of the study has been defined as those patients who receive NI-0501 after having failed conventional HLH therapy or having shown intolerance to it.

To further support the evidence of NI-0501 efficacy and safety, data obtained in the long-term follow-up NI-0501-05 study (see protocol synopsis in Appendix 7.3) represent an integral part of the statistical analyses, and are therefore considered in the present SAP.

Briefly, all patients enrolled in the NI-0501-04 study, after either completing or having discontinued the study, are invited to participate in the long-term follow-up study NI-0501-05 in order:

i) to assess long-term outcome and survival after NI-0501 treatment and HSCT (if performed)

ii) to monitor the long-term safety profile of NI-0501

iii) to complete the characterization of NI-0501 PK profile

NI-0501 treatment can be continued in the context of the long-term follow-up study NI-0501-05, upon the request of the Investigator in case of the need to delay the schedule for transplantation for reasons unrelated to the administration of NI-0501, provided that a favorable benefit/risk has been established for the patient.

The planned statistical methods and analyses have been selected taking into account, where applicable, relevant sections of the International Conference on Harmonisation (ICH) E9 Guideline "Statistical Principles for Clinical Trials," and the ICH E3 Guideline "Structure and Content of Clinical Study Reports."

This statistical analysis plan (SAP) is based on the latest version of protocol NI-0501-04 (EU v6.0 and US v5.1) and NI-0501-05 (EU v2.1 and US v2.2).

1.2. Objectives of Statistical Analysis

The objectives of the study NI-0501-04, as stated in the protocol, are:

- To determine the safety and tolerability profile of multiple IV administrations of NI-0501
- To determine the efficacy and benefit/risk profile of NI-0501 in HLH patients
- To describe the PK profile of NI-0501 in HLH patients
- To define an appropriate NI-0501 therapeutic dose regimen for HLH
- To determine the pharmacodynamic (PD) effects of NI-0501 on circulating levels of total IFNγ and biomarkers of its neutralization, namely CXCL9 and CXCL10
- To determine the PD effects of NI-0501 on other biomarkers, e.g., sCD25
- To assess the immunogenicity of NI-0501

This SAP is designed to outline the methods to be used in the analysis of study data. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the result sections of the final clinical study report (CSR) for this trial. All analyses outlined in this SAP will apply to the combined data from both the US and EU versions of the protocol (including data from the long-term follow-up NI-0501-05 protocols, as above mentioned); likewise, references to the "study" will be interpreted as referring to the clinical trial regardless of protocol version.

This SAP will also outline changes in the analytical plan relative to the one initially described in the study protocol, as applicable.

A separate analysis plan and report will be generated to describe the PK and PD analyses, and in particular to address the following objectives:

- To describe the PK profile of NI-0501 in HLH patients
- To determine the PD effects of NI-0501 on circulating levels of total IFNγ and biomarkers of its neutralization, namely CXCL9 and CXCL10
- To determine the PD effects of NI-0501 on other biomarkers, eg, sCD25

2. STUDY DESIGN

2.1. Synopsis of Study Design

The synopsis of the NI-0501-04 protocol (EU v6.0 26 February 2016; US v5.1 24 March 2016) is presented in Table 2-1.

The synopsis of the NI-0501-05 protocol is presented in Appendix 7.3.

Table 2-1: Study Synopsis

Title:	A Phase 2/3 open-label, single arm, multicenter study to assess safety, tolerability, pharmacokinetics and efficacy of intravenous multiple administrations of NI-0501, an anti-interferon gamma (anti-IFNγ) monoclonal antibody, in pediatric patients with primary Hemophagocytic Lymphohistiocytosis (HLH)
Sponsor:	Novimmune SA, Switzerland
Study Type, Phase and Design:	- Interventional Phase 2/3 study. - Open-label, single arm, multicenter study. - NI-0501-04 study is performed both in the US and in Europe according to twin protocols called NI-0501-04 (US-P-IND#111015) and NI-0501-04 (EudraCT#2012-003632-23), respectively.
Study Objectives:	- To determine the safety and tolerability profile of multiple intravenous (IV) administrations of NI-0501. - To determine NI-0501 efficacy and benefit/risk profile in HLH patients. - To describe the pharmacokinetics (PK) profile of NI-0501 in HLH patients. - To define an appropriate NI-0501 therapeutic dose regimen for HLH. - To assess the immunogenicity of NI-0501.
Study Population:	- Primary HLH patients. - Patients can be naïve to HLH treatment (first line patients), or may have already received conventional HLH therapy (second line patients) without having obtained a satisfactory response according to the treating physician or having shown signs of intolerance to it. - Patients who receive NI-0501 as second line treatment for HLH will represent the pivotal cohort of the study.
Main Inclusion Criteria:	- Primary HLH patients of both genders, up to and including 18 years at diagnosis of HLH. - Presence of active disease as assessed by the treating physician. - Patient (if ≥ 18 years old), or patient's legally authorized representative(s) must have signed informed consent. - Having accepted contraceptive measures whenever necessary

Exclusion Criteria:	- Diagnosis of secondary HLH consequent to a proven rheumatic or neoplastic disease. - Body weight < 3 kg. - Patients treated with: ▪ any T-cell depleting agents (such as anti-thymocyte globulin [ATG], anti-CD52) during the previous 2 weeks prior to screening. ▪ any other biologic drug within 5 times their defined half-life period (except for rituximab in case of documented EBV infection). - Active mycobacteria, *Histoplasma Capsulatum, Shigella*, *Salmonella*, *Campylobacter* and *Leishmania* infections. - Evidence of past history of tuberculosis or latent tuberculosis. - Positive serology for HIV antibodies, hepatitis B surface antigen or hepatitis C antibodies. - Presence of malignancy. - Patients who have another concomitant disease or malformation severely affecting the cardiovascular, pulmonary, liver or renal function. - History of hypersensitivity or allergy to any component of the study regimen. - Receipt of a live or attenuated live (including BCG) vaccine within the previous 12 weeks from screening. - Pregnant or lactating female patients.
Study Drug:	- NI-0501 is a fully human IgG1 monoclonal antibody (mAb) directed against human IFNγ.
Dosing Regimen & Frequency of Administration:	- NI-0501 will be administered by IV infusion over a period of one hour at an initial dose of 1 mg/kg. - Infusions will be performed every 3 days until Study Day 15 (SD15) (infusion #6), and twice per week thereafter. - NI-0501 dose increase to 3 mg/kg will be possible according to pre-defined criteria guided by clinical and laboratory response in each patient at any time during the study. - After a minimum of 2 infusions at 3 mg/kg if, upon re-assessment, the same clinical and laboratory criteria qualifying the patient to receive 3 mg/kg of NI-0501 are found to still apply, the dose of NI-0501 may be increased to 6 mg/kg for up to four infusions, with a regular monitoring of the clinical and laboratory HLH parameters. - Based on the evolution of these parameters, the dose of NI-0501 may either *i*) be decreased back to 3 mg/kg, or *ii*) remain at 6 mg/kg for additional infusions (or be increased above 6 mg/kg), if PK and PD evidence indicates excessively high IFNγ production and, consequently, fast NI-0501 elimination. - Dose increase may occur any time during the study, if the clinical and laboratory criteria are met.

Treatment Duration:	- NI-0501 administration is foreseen for 8 weeks. After this time period, the conditioning regimen in preparation for Hematopoietic Stem Cell Transplantation (HSCT) might be initiated. - The anticipated duration of treatment can be shortened, although not to less than 4 weeks, if the patient's condition and donor availability allow the performance of a transplant. - In the event that an appropriate donor has not been identified by Week 8 or in case of the need to delay the schedule for transplantation for reasons unrelated to the administration of NI-0501, NI-0501 treatment can be continued, upon the request of the Investigator, in the context of the long-term follow-up study NI-0501-05, provided that a favorable benefit/risk has been established for the patient.
Background Therapy & Concomitant Medication:	- NI-0501 will be administered on a background of dexamethasone, which can be tapered depending on patient condition. - Patients will receive prophylactic treatment for *Pneumocystis jiroveci*, fungal and *Herpes Zoster* virus infection from the day before initiation of NI-0501 treatment until the end of the study. - Cyclosporin A (CsA) can be continued if already being administered to the patient prior to screening. CsA can be withdrawn at any time, upon judgment of the Investigator. CsA is not to be introduced *de novo* during the course of the study once NI-0501 administration has started. - If the patient is receiving intrathecal methotrexate and glucocorticoids at the time of NI-0501 treatment initiation, this treatment will be continued as required. - IV immunoglobulins (IVIG) are only allowed as replacement treatment in case of a documented immunoglobulin deficiency. - Analgesic treatment, transfusion of blood products, electrolyte and glucose infusions, antibiotics, anti-fungal and anti-viral treatment and general supportive care are allowed. - Vaccination with a live or attenuated (including BCG) vaccine must be avoided during the whole study including the 4-week follow-up period. - Additional HLH treatments may be allowed in case of unsustained or limited HLH improvement once the maximum NI-0501 dose level is achieved. ▪ Unsustained HLH Improvement: Patients who are unable to maintain at least 50% improvement from baseline for 3 HLH parameters (see Table 2-2). At least two consecutive measurements must document the loss of HLH improvement. ▪ Limited HLH Improvement: Less than 50% change from baseline in a minimum of 3 HLH clinical and laboratory criteria. Etoposide should be administered as additional HLH treatment, unless clear evidence of lack of response or intolerance to the drug is derived from previous medical history. In this circumstance, the Investigator may propose an alternative agent which requires to be approved by the Data Monitoring Committee.

Sample Size:	- Sample size is estimated for the pivotal cohort of the study, ie, patients receiving NI-0501 in second line. - A minimum of 28 evaluable second line patients will be enrolled in the study. Sample size calculation is based on the primary efficacy endpoint of "Overall Response Rate." Assuming an ORR of 70%, the study will have 90% power to show a significant improvement above 40% using an exact binomial test at a one-sided significance level of 2.5%.
Number of Sites and Recruitment Duration:	- It is estimated that in the US approximately 8 sites will participate in this study. The time needed to complete enrolment of the required number of second line patients, in this rare population, is estimated to be approximately 1 year. - A twin protocol is actively recruiting in European countries. The recruitment will be competitive across all US and European sites.
Study Duration and Study End Definition:	- After the treatment period, or, in any case, at treatment discontinuation, patients will enter a follow-up period of 4 weeks (short-term follow-up). - End of the study is defined as last patient last visit. - A separate long-term follow-up study (NI-0501-05) will enroll all patients who will have received at least one dose of NI-0501 and signed informed consent.
Study Safety Monitoring and Stopping Rules:	- An independent Data Monitoring Committee (DMC) composed of relevant Experts (pediatric onco-hematologists, pediatric immune deficiency/infectious disease specialists, a bio-statistician and a specialist in ethics) will oversee the study conduct, reviewing data generated both in the US and in Europe. - The main DMC responsibility is to review all safety and relevant efficacy data as they are generated on an on-going basis, with the objective of determining the benefit/risk profile of NI-0501 treatment for HLH patients and ensuring that no patient is exposed to unnecessary risks. - The DMC can recommend treatment discontinuation for individual patients as well as temporarily or permanently stopping the entire study. Predefined stopping rules will guide the DMC's review process. - Patients withdrawn from the study will receive rescue therapy, according to the standard of care at the site. - A patient, his/her representative or the Investigator can decide at any time to withdraw a patient from the study. This decision will have no impact on the patient's care.
Efficacy Endpoints:	Evolution of clinical signs (fever, splenomegaly, CNS symptoms) and laboratory parameters (CBC, fibrinogen, ferritin, sCD25 levels), which characterize the disease, will be used to assess the achievement of response and time to response. Primary efficacy endpoint: - Overall Response Rate, ie, achievement of either Complete or Partial Response or HLH Improvement, at End of Treatment (EOT), as defined in Table 2-2.

	Secondary efficacy endpoints: - Time to Response at any time during the study - Durability of Response, i.e., maintenance of response achieved any time during the study until EOT and beyond (including data collected in the long-term follow-up study NI-0501-05). - Number of patients able to reduce glucocorticoids by 50% or more of baseline dose. - Number of patients able to proceed to HSCT, when deemed indicated. - Survival at Week 8 (or EOT) and at the end of the study [Long-term survival (in particular D+30 and D+100 post-HSCT survival) will be assessed in the context of long-term study NI-0501-05]. - Serum concentration of NI-0501 to determine NI-0501 pharmacokinetic (PK) profile. - Determination of pharmacodynamic (PD) effects (levels of circulating total IFNγ and markers of its neutralization, namely CXCL9 and CXCL10). - Determination of other biomarkers, eg, sCD25, IL-10.
Safety Endpoints:	Safety parameters to be collected and assessed: - Incidence, severity, causality and outcomes of Adverse Events (serious and non-serious), with particular attention being paid to infections. - Evolution of laboratory parameters such as complete blood cell count (CBC), with a focus on red cells (hemoglobin), neutrophils and platelets, liver tests, renal function tests and coagulation. - Number of patients withdrawn for safety reasons. Other parameters: - Level (if any) of circulating antibodies against NI-0501 to determine immunogenicity (ADA).
Statistical Analysis:	- The primary endpoint Overall Response Rate will be evaluated using the exact binomial test at the one-sided 0.025 level. - Time to Response, durability of Response and Survival time will be presented using Kaplan-Meier curves with medians calculated if estimable. Two-sided 95% confidence intervals will be calculated for the median for each of these endpoints. - Additional endpoints based on binary outcomes including number of patients who reduce glucocorticoids by 50% or more, and number of patients able to proceed to HSCT will be converted to proportions and associated 2-sided 95% confidence intervals calculated. - Statistical significance in terms of p-values will only be obtained for the primary endpoint. All other endpoints will be viewed as supportive for the primary endpoint and as a consequence no formal hierarchy of endpoints will be declared.

2.2. Randomization Methodology

Not applicable as this is a single arm study.

2.3. Stopping Rules and Unblinding

Pre-defined stopping rules have been set in the study protocol (protocol section 10).

Briefly, the patient or patient's representative could withdraw at any time consent to study participation, as well as the Investigator could decide at any time during the study to discontinue the treatment for an individual patient based on his/her own medical judgment, taking into account the individual benefit risk ratio.

The protocol defines the situations that may trigger an immediate decision to permanently discontinue treatment in a patient, because of:

i) safety reasons, namely the occurrence of a life-threatening SAE during NI-0501 treatment assessed by the Investigator to be related to NI-0501 (with guidance from the Data Monitoring Committee [DMC] if needed).

ii) lack of efficacy, i.e., no response or lack of improvement observed even after administration of an additional HLH treatment concomitantly to NI-0501.

The study conduct has been overseen on an ongoing basis by an independent DMC composed of relevant Experts (pediatric onco-hematologists, pediatric immune deficiency/infectious disease specialists, a biostatistician and a specialist in ethics).

The main DMC responsibility has been to review safety and relevant efficacy data as they were generated, with the objective of determining the benefit/risk profile of NI-0501 treatment in the patients enrolled in the study, thus ensuring that no patient was exposed to unnecessary risks.

The DMC could recommend treatment discontinuation for individual patients as well as temporary study suspension or study termination.

Monthly meetings have been generally held during the period of the study, and additional ad hoc DMC meetings have been convened whenever required for urgent data presentation and discussion.

Unblinding does not apply as the study is open label.

2.4. Study Procedures

The schedule of assessments of the NI-0501-04 study, as outlined in the protocol, is provided in Appendix 7.4.

2.5. Efficacy and Safety Variables

2.5.1 Primary Efficacy Endpoints

The primary efficacy endpoint of the NI-0501-04 study is:

- Overall Response, i.e., achievement of either Complete or Partial Response, or HLH Improvement, at End of Treatment (EOT NI-0501-04 – Primary efficacy time-point).

Criteria for the definition of Response are reported in Table 2-2. A more detailed specification for implementation of these criteria to derive Overall Response is presented in Appendix 7.1.

Table 2-2: Definition of Response

Complete Response	Complete Response is adjudicated if: - No fever = body temperature < 37.5°C - Normal spleen size confirmed by abdominal ultrasound whenever possible - No cytopenia = Absolute Neutrophil Counts ≥ $1.0x10^9$/L and platelet count ≥ $100x10^9$/L [absence of G-CSF and transfusion support must be documented for at least 4 days to report no cytopenia] - No hyperferritinemia = serum ferritin level is < 2000 µg/L - No evidence of coagulopathy, i.e., normal D-Dimer and/or normal (> 150 mg/dL) fibrinogen levels - No neurological and CSF abnormalities attributed to HLH - No sustained worsening of sCD25 (as indicated by at least two consecutive measurements that are > 2-fold higher than baseline)
Partial Response	Partial Response is adjudicated if: - At least 3 of the HLH clinical and laboratory abnormalities (including CNS abnormalities) meet the above-mentioned criteria for "Complete Response". In the case of "reactivated patients" who enter the study with 3 abnormal HLH features, at least 2 criteria should meet the definition given - There is no progression of other aspects of HLH disease pathology (e.g., jaundice, liver size, oedema, CNS clinical alterations)
HLH improvement	- Normalization or Improvement (>50% change from baseline) of at least 3 HLH clinical and laboratory abnormalities (including CNS involvement). In the case of "reactivated patients" who enter the study with 2 abnormal HLH features, a change from baseline greater than 50% for both will define HLH as improved.

2.5.2 Secondary Efficacy Endpoints

The following secondary efficacy endpoints will be assessed as detailed in Section 4.6:

- Time to First Response (any time during the study)
- Durability of First Response until EOT 04 (overall and by continent)
- Cumulative duration of response (overall and by continent)
- Overall Response at Week 2 of treatment
- Time to HSCT: Time to HSCT is defined as the time from the date of first dose to the date of HSCT.
- Number of patients who reduce glucocorticoids by 50% or more of the baseline dose at EOT 04 and at EOT 04/05
- Number of patients able to proceed to HSCT, when deemed indicated. Listing will be provided for type of performed HSCT (e.g., donor type, stem cell source), conditioning regimen and GVHD prophylaxis administered (including data collected in the long-term follow-up study NI-0501-05)
- Post-HSCT outcome measures including Post-HSCT engraftment rates, incidence of Post-HSCT acute and chronic GvHD, and Post-HSCT relapse rates

- Assessment of Response by the Investigator at the per-protocol time points (i.e., SD15, SD27 and EOT 04)
- Overall Survival (Overall and by continent)
- Overall Survival post Week 2 by Responder Status
- Survival to HSCT (Overall and by continent)
- Survival post HSCT (Overall and by continent)
- Event-free survival

2.5.3 Exploratory Efficacy Endpoints

- Best overall response rate and time to best overall response up to EOT 04 and up to EOT 04/05
- Overall response at start of conditioning (pre-Conditioning) among patients who proceeded to HSCT

2.5.4 Safety Variables

Safety and tolerability of NI-0501 will be assessed as follows:

- Incidence, severity, causality and outcomes of AEs (serious and non-serious), with particular attention being paid to infusion-related reactions (IRRs) and infections
- Evolution of relevant laboratory parameters
- Number of patients withdrawn due to AE
- Level (if any) of circulating antibodies against NI-0501 to determine immunogenicity; i.e., the development of anti-drug antibodies (ADAs)

2.6. Pharmacokinetics and Pharmacodynamics Variables

Pharmacokinetics analyses, as well as PK/PD characterization, will be performed through PK/PD modelling described in a separate analysis plan and report.

In the present SAP, the following PD parameters are considered for the purpose of descriptive statistics only:

- Levels of serum free IFNγ at pre-dose on SD0 (i.e., before start of NI-0501 treatment)
- Levels of serum total IFNγ (free + bound) at subsequent time points after initiation of NI-0501 treatment
- Levels of serum sCD25, CXCL-9 and CXCL-10

3. PATIENT POPULATIONS

3.1. Population Definitions

The following patient populations will be considered for presentation and analysis of data:

1. **All Treated Analysis Set:** all patients who received any part of an infusion of the study drug.
2. **All Treated Evaluable Analysis Set:** patients who are evaluable for efficacy according to the criteria described in Section 3.2.
3. Within the All Treated sets, the following populations are further defined:
    a. **2nd Line All Treated Analysis Set (Pivotal Cohort):** patients in the All Treated analysis set who have already received conventional HLH therapy prior to enrolment in the study, therefore are treated with NI-0501 in 2nd line. This analysis set will be used for the **primary efficacy analysis**.
    b. **2nd Line Evaluable Analysis Set:** patients in the 2nd line All Treated set who are evaluable for efficacy according to the criteria described in Section 3.2. This analysis set will be **supportive to the primary efficacy analysis.**

Efficacy will be analyzed in all patient populations.

The **primary efficacy analysis** will be performed on the 2nd line All treated Analysis Set with supportive evidence derived from the 2nd line Evaluable analysis set. Further details on identifying 2nd line patients are provided in Appendix 7.2.

The **primary safety analysis** will be performed on the **All Treated Analysis Set** (as above defined) on all data collected prior to AND after conditioning for HSCT.

Analyses and summaries with the relevant analysis sets are described in Table 3-1.

For the analysis of some secondary efficacy endpoints and safety analyses, data from the NI-0501-05 study in the defined patient populations will also be considered.

Table 3-1: Patient Populations for Each Analysis/Summary

	Patient Populations			
Analyses	**2nd Line All Treated Analysis Set Pivotal Cohort**	**2nd Line Evaluable Analysis Set**	**All Treated Analysis Set[3] (1st and 2nd Line)**	**All Treated Evaluable Analysis Set (1st and 2nd Line)**
Patient Disposition	✓	✓	✓	✓
Demographics, Baseline	✓	✓	✓	✓
Past and Concomitant Therapies	✓	✓	✓	✓
Primary Efficacy Endpoint	✓ (primary analysis)	✓ (supportive)	✓	✓
Secondary Efficacy Endpoints	✓	✓	✓	✓
Sensitivity analyses[1]	✓	✓		
Compliance and Exposure	✓		✓	
Safety: AEs, SAEs, Lab tests	✓		✓	
Safety: Vital Signs, Physical Examination, ECG[2]	✓		✓	

[1] *To be performed on primary efficacy endpoint only.*
[2] *A separate ECG report will be provided by a specialized CRO. Therefore, the analysis and statistical methods are not described in this SAP.*
[3] *This set will be also used for the Primary Safety analysis.*

For additional subpopulations and corresponding analyses, please refer to Section 4.2.7.

3.2. Criteria for Evaluability and Protocol Deviations

Patients will be considered "evaluable" if:

- NI-0501 treatment is administered for at least 3 consecutive infusions
- No exclusion criteria have emerged after enrolment, that can significantly impact on efficacy evaluation
- No protocol deviations have occurred, that can significantly impact on efficacy evaluation

Adjudication to the "evaluable" Analysis Set will be made jointly by the two physicians of the Independent DMC and the US and EU Principal Investigators of the study, as required based on preliminary review of patient data.

Protocol deviations will be determined by a review of the data and information documented by the study CRAs prior to database lock.

Protocol deviations will be medically reviewed. Protocol deviations, especially related to occasional lack of adherence to the schedule of assessments or visit timings (primarily

attributable to optimization in the clinical management of this fragile children population), will not be presented.

Important deviations will be summarized in a table and presented by data listing, considering at minimum the following categories:

- Eligibility: patients entering the study although not meeting all eligibility criteria (including patients in whom any of the exclusion criteria has been diagnosed after treatment start)
- Co-medication: non-adherence to protocol with regard to administration of concomitant medication (including non-allowed medication that would significantly impact efficacy evaluation and interruption of required prophylaxis for more than 1 week, unless clinically justified)
- Study drug: deviations re. treatment dose (i.e., a daily dose >25% higher or lower than the planned dose) and frequency of administrations (i.e., interval between NI-0501 infusions >2 days longer or shorter than what indicated in the corresponding protocol version)
- Assessment: deviations re. schedule of assessments (e.g., more than 2 missing consecutive examinations required per protocol or assessment performed at time-points different from those required by the protocol)
- Others: reasons other than protocol deviations impacting on patient evaluability

An assessment of the potential impact on study data analysis and subject's well-being (e.g. major, minor) will also be included.

4. STATISTICAL METHODS

4.1. Sample Size Justification

Sample size estimation has been done for the pivotal cohort of the study, i.e., pHLH patients receiving NI-0501 as second line treatment of HLH.

A minimum of 28 evaluable patients treated with NI-0501 in second line will be enrolled in the study. Sample size calculation is based on the primary efficacy endpoint of "Overall Response." Assuming an Overall Response Rate of 70%, the study will have 90% power to show a significant improvement above 40% using an exact binomial test at a one-sided significance level of 2.5%.

Due to the rarity of pHLH, the recruitment is competitive across all EU and US sites in order to gather data in a reasonable timeframe.

4.2. General Statistical Methods and Data Handling

4.2.1 General Methods

Statistical methods will focus on summarizing the data collected using appropriate graphical and tabular presentations and on generation of inferential summaries.

For measurements of continuous endpoints, summary statistics will include n, mean, median, standard deviation, minimum and maximum values. Mean, standard deviation, and median will be presented with one more decimal place compared to the raw data, minimum and maximum will be presented with same number of decimal places as the raw data.

For categorical variables, summary tabulations of the number and percentage within each category (with a category for missing data) of the parameter will be presented. Percentages will be rounded to one decimal place. Therefore, there may be cases where for instance the total of the percentages does not exactly equal 100%. In case confidence intervals are provided, they will be shown with 1 decimal place.

P-values will be provided with three decimal places.

All summarized data will be listed in addition to the analyses and summaries described below. Any relevant data present in the final locked database which is not summarized or analyzed according to the plans below will be listed.

4.2.2 Data Conventions

The following conversion factors will be used to convert days to months or years, or Fahrenheit to Celsius, where applicable:

- 1 month = 30.4375 days
- 1 year = 365.25 days
- 1 week = 7 days
- °C = (°F - 32)/1.8000

Study Day 0 (SD0) is the day when first NI-0501 infusion is performed.

Baseline assessment is defined as the last assessment performed prior to the first NI-0501 infusion (including SD0 prior to infusion).

Screening assessment is defined as the assessment performed during the screening period (up to 12 days prior to SD0), depending on data availability (closest values to ICF signature will be considered).

For the purpose of the primary efficacy analysis (see Section 4.6.1.1), End of treatment (EOT 04) is defined as the visit performed 3 days after the last NI-0501 infusion in the NI-0501-04 study. A -3/+5-day window is allowed provided it is before start of conditioning for HSCT.

For patients continuing treatment in NI-0501-05 study, EOT 04/05 is defined as the visit performed 3 days after the last NI-0501 infusion in the NI-0501-05 study, with -3/+5-day window provided it is before start of conditioning.

Additionally, a -3/+3-day window will be considered for sensitivity analyses (see Section 4.6.1.2).

4.2.3 Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software (Version 9.2 or later), unless otherwise noted.

Medical History and adverse events (AEs) will be coded using current Medical Dictionary for Regulatory Activities version at the time of data cut-off date (MedDRA version 22.0).

Concomitant medications will be coded using World Health Organization (WHO) Drug dictionary 2019 version, Level 4.

4.2.4 Methods of Pooling Data

Data from the NI-0501-04 and the NI-0501-05 studies will be combined for some analyses as specified elsewhere in the SAP.

4.2.5 Adjustments for Covariates

No formal statistical analysis that adjusts for possible covariate effects is planned.

4.2.6 Multiple Comparisons/Multiplicity

No multiplicity adjustment is considered.

4.2.7 Subpopulations

Selected efficacy analyses and safety analysis will be repeated on other sub-group(s) of the All Treated Population as below:

- 1st line all treated patients:
    - Primary efficacy endpoint: Overall Response Rate Sensitivity Analysis 1, 2, 3 and 4 as described in Section 4.6.1
    - Secondary efficacy endpoints: Time to First Response, Durability of First Response until EOT 04, Cumulative duration of response, Overall Response at Week 2 of treatment, Binary secondary endpoints described in Section 4.6.2 ,

Assessment of Response by Investigator, Survival Pre/Post HSCT, Event Free Survival Post HSCT

- Evolution of biomarkers over time
- Safety endpoints: Overall Summary of AEs, TEAEs, Serious TEAEs, Severe TEAEs, TEAEs related to infections during Pre-conditioning, TEAE related to confirmed IRR

- Geographical region (US vs EU):
  - Demographics
  - Key efficacy results including Overall Response Rate, Durability of First Response until EOT 04, Cumulative duration of response, Overall Survival, and Survival Pre/Post HSCT
- Subgroup of patient who undergo HSCT: Overall Response Rate at start of conditioning (pre-Conditioning), presented separately for 1st line, 2nd line and all treated patients.

4.2.8 Withdrawals, Dropouts, Loss to Follow-up

Subjects who are withdrawn or discontinue from the study will not be replaced.

4.2.9 Missing, Unused, and Spurious Data

Imputation of missing data for defining efficacy endpoints is provided in Section 4.6.1.

Partially missing dates for adverse events (AEs) will be imputed as follows. Of note, imputation of missing/partial AE date will be done only to identify treatment emergent AEs.

AE onset dates

- Partially missing onset dates will be imputed as follows:
  - When only Day is missing:
    - If Month & Year of the onset date are the same as Month & Year of the first infusion date, the imputed onset date will be imputed as the minimum of the first infusion date and the AE resolution date (imputed if needed).
    - Otherwise, the missing day will be replaced by "1."
  - When Day & Month are missing:
    - If Year of the onset date is the same as Year of the first infusion date, the imputed onset date will be imputed as the minimum of the first infusion date and the AE end date (imputed if needed).
    - Otherwise, the missing Day & Month will be replaced by "01JAN."
- Complete missing onset dates for AEs will be imputed by the first infusion date and the AE will be considered as treatment emergent, unless the end date of the AE (imputed if needed) or the end year of the AE (if day and month are missing) is entered and is before the first infusion date.

AE end dates (for non-ongoing events)

- If day only is missing, incomplete end dates will be replaced by the last day of the month, if not resulting in a date later than the date of patient's death. In the latter case the date of death will be used to impute the incomplete end date.
- If day & month are missing, day & month will be replaced by the 31DEC, if not resulting in a date later than the date of patient's death. In the latter case the date of death will be used to impute the incomplete end date.
- In all other cases the incomplete end date will not be imputed.

Medication dates

Partially missing dates for medication will be imputed as follows. Of note, imputation of missing/partial medications date will be done only to help differentiating between prior vs. concomitant medication.

- For a medication recorded in the NI-0501-04 study, if the start date of the medication is unknown (i.e., complete missing date) and there is a record indicating that medication is not ongoing (although end date is unknown), the medication will be considered prior to enrolment.
- If the start date of the medication is unknown (i.e., complete missing date) and there is no end date or any otherwise evidence that medication is not ongoing, the worst-case scenario will be assumed. The medication will be considered as both a prior medication and a concomitant medication.
- If the month and the day of the start date of the medication are missing, the month and the day will be imputed to January 1st of the year specified.
- If the day of the start date of the medication is missing and there is no end date, the day will be imputed to the first day of the month specified.
- If the month and the day of the end date of the medication are missing, the month and the day will be imputed to December 31st of the year specified, unless there is evidence that the medication is not ongoing in the NI-0501-04 study (although complete end date is unknown). In such circumstances the medication will be considered as prior to enrolment.
- If the day of the end date of the medication is missing, the day will be imputed to the last day of the month specified, unless there is evidence that the medication is not ongoing in the NI-0501-04 study (although complete end date is unknown). In such circumstances the medication will be considered as prior to enrolment.

No other dates will be imputed, unless otherwise specified. In data listings, the original incomplete, missing or partial dates (not the imputed dates) will be presented.

Normal ranges

For assessments where normal ranges are not routinely provided by the site laboratory (e.g. WBC differential), missing ranges from the literature will be imputed as appropriate.

4.2.10 Visit Windows

Besides the visit windows allowed per protocol, time windows are considered in case of missing data for defining endpoints (see Section 4.6.1 and Section 4.6.2).

For the assessment of endpoints at pre-defined time-points, time windows are summarized in Table 4-1.

Table 4-1: Time Windows for Endpoint Assessment at Pre-defined Time Points

Visit	Time Window used in Analyses
Screening	During the 1-wk screening, considering the closest value to ICF signature if more than one value is available
Baseline	Last value prior to or at SD0 (pre-infusion)
SD14 (week 2)	SD11 to SD17
SD28 (week 4)	SD25 to SD31
SD42 (week 6)	SD39 to SD45
NI-0501-04 EOT	SDxx-3 to SDxx+5 (provided before start of conditioning) SDxx-3 to SDxx+3 (additional time window for efficacy sensitivity analysis, see Section 4.6.1.2)
NI-0501-04/05 EOT	SDxx-3 to SDxx+5 (provided before start of conditioning) SDxx-3 to SDxx+3 (additional time window for efficacy sensitivity analysis, see Section 4.6.1.2)
Pre-conditioning	Last values prior the start of first conditioning treatment (not earlier than 3 days)
Pre-HSCT	Last value prior to HSCT (not earlier than 3 days)
Short-term Follow-up visit (NI-0501-04)	SDxx-3 to SDxx+3
Follow-up visit till Day+30 post-HSCT (or after last NI-0501 infusion)	SDxx-3 to SDxx+3
Follow-up visit till Day+60 post-HSCT (or after last NI-0501 infusion)	SDxx-14 to SDxx+14
Follow-up visit till Day+100 post-HSCT (or after last NI-0501 infusion)	SDxx-14 to SDxx+14
6-months and 12-months post HSCT (or after last NI-0501 infusion)	SDxx-30 to SDxx+30

In the case that a parameter is not available on the specified study day, the visit windowing is to be applied. If there is more than one observation during the visit window, a value will be selected as follows: the closest value (within the visit window specified in Table 4-1) to the actual study day in question will be chosen first. In the case that there are 2 equidistant values to the study day of interest, the observation that is after the study day in question will be selected.

For example, if there is a missing value on SD14 but a value available on SD13 and SD15, the value from SD15 will be selected. In the case of 2 or more measurements on the same date, the earliest post 4 am measurement will be selected.

Vital signs and physical exam summaries will be presented using pre-defined visits as follows: Screening, Baseline, SD0 (measurements surrounding first infusion as detailed in Section 4.8.6), SD3, SD6, SD9, SD12, SD15, and subsequently on SD21, SD28, SD35 (etc., weekly with +/-3 days windows) until EOT 04/05.

Laboratory safety tables by time point will be presented for the following pre-defined visits: Screening, Baseline, SD0, SD1, SD2, SD3, SD5, SD6, SD8, SD11 (no windowing) and SD14, SD21, SD28, SD35 (etc., weekly with +/-3 days windows) until EOT04/05.

Post EOT 04/05 laboratory data will be presented separately for patients who had HSCT and patients who did not have HSCT:

1. For patients who had HSCT, laboratory parameters will be presented for the following time points: Pre-conditioning visit, Pre-HSCT visit and followed post HSCT based on number of study days since date of HSCT as follows: SD7P, SD14P, SD21P, SD30P, SD60P, SD100P, and 6-months (6MONP) and 12-months post HSCT (12MONP).
2. For patients who did not have HSCT, laboratory parameters will be presented for the following time points post EOT 04/05, defined by number of study days since date of last infusion of NI-0501 (i.e., post treatment): SD7PT, SD14PT, SD21PT, SD30PT, SD60PT, SD100PT, 6-months (6MONPT) and 12-months post last infusion (12MONPT).

4.3. Timing of Analyses

In September 2015, an unplanned, informal descriptive analysis was conducted to prepare for a requested regulatory data submission prior to the database lock, in the context of application to the Food and Drug Administration (FDA) for Breakthrough Therapy designation and to the European Medicines Agency (EMA) for PRIME designation. This analysis was done on a partially cleaned database when a total of 11 patients had received at least one infusion of the study drug. Data were also presented for four additional patients treated with the study drug in compassionate use and not enrolled in the NI-0501-04 study. The analysis included a summary in the All Treated Population of demographics, HLH diagnostic criteria and laboratory parameters (including improvement/normalization).

The primary efficacy and safety analyses were performed on a database cut-off date of 20 July 2017 for regulatory application purposes, including Biologics License Applications (BLA) and Marketing Authorization Applications (MAA) submissions with the FDA and EMA respectively. An interim CSR was provided, as the study remained open and patients continued in follow-up.

A follow-up analysis will be performed to support the final CSR post database lock for the NI-0501-04 study, to occur after the last patient has completed last study visit.

This analysis will include long-term follow-up data gathered in the NI-0501-05 study up to 31 January 2019.

4.4. Patient Disposition

A tabulation of patient disposition will be provided, including:

- The number of patients screened
- The number of screen failures and reason for screen failures
- The number of treated patients
  - The number of 1st line patients
  - The number of 2nd line patients
- The number of patients in the All Treated Analysis Set
- The number of patients in the 2nd Line All Treated Analysis Set (Pivotal Cohort)
- The number of patients in the All Treated Evaluable Analysis Set
- The number of patients in the 2nd Line Evaluable Analysis Set
- The number of treated patients who completed NI-0501-04 study
  - Patients completing the study are the ones who
    - completed NI-0501 treatment, ie received a minimum of 4 weeks of treatment (from SD0 to EOT) unless discontinued subsequently due to lack of efficacy or safety reason
      and
    - completed the 4-week short-term follow-up or continued treatment in the NI-0501-05 study
- The number of treated patients who withdrew prior to completing NI-0501-04 study
  - The reasons for withdrawal (percentages will be based on patients who withdrew prior to completing NI-0501-04 study)
- The number of patients enrolled in NI-0501-05 study
- The number of patients receiving NI-0501 treatment in the NI-0501-05 study
- The number of patients who completed NI-0501-05 (i.e. patients who performed the visit at 1 year after HSCT or after last NI-0501 infusion if HSCT was not performed). Patients still ongoing in NI-0501-05 study will be reported
- The number of patients who withdrew prior to completing NI-0501-05 study
  - The reason for withdrawal (percentages will be based on patients who withdrew prior to completing the NI-0501-05 study)

Furthermore, the number of patients in the Analysis Set defined in Table 3-1 will be summarized by country and center.

A by-patient listing of study completion information, including the reason for premature study withdrawal, if applicable, will be presented.

A by-patient listing of eligibility criteria not met by patient will be presented.

A by-patient listing of protocol deviations, as described in section 3.2, will be presented.

4.5. Demographic and Baseline Characteristics

Demographic and baseline data will be summarized in all populations as defined in Table 3-1.

4.5.1 Demographics and Baseline Characteristics

The following information will be provided:

- **Summary of patient demographics**
    - Age at informed consent (years)
    - Sex (Male, Female)
    - Race and Country of Origin (as available)

  Of note, age at informed consent is derived as duration between date of informed consent and date of birth

  The above variables will also be presented by continent (US vs EU)

- **Summary of other patient characteristics**
    - Weight (kg)
    - Height (cm)
    - Body surface area [BSA] (m2)

- **Summary of HLH disease characteristics at diagnosis**
  [Of note, HLH diagnosis in 2nd line patients is antecedent to study enrolment; hence HLH disease characteristics will differ from those at study baseline]
    - Age at diagnosis (years), derived as duration between date of diagnosis and date of birth
    - Number of HLH criteria met by a patient
    - Number of patients meeting each HLH criteria
    - Number of patients previously treated for HLH

- **Summary of previous HLH treatments**
    - Treatments administered, including type of drug (of note, for some patients, information may be available on treatment regimen only, e.g. HLH-94, HLH-2004)
    - For patients who achieved a Response with conventional HLH treatment, the following will be summarized
        - Type of Response achieved as reported by the investigators, including complete response, partial response and incomplete response
        - Time to Response defined as (date of response achieved - date of HLH diagnosis +1). If time to response is longer than 60 days, it will be censored as 60 days for summaries

- For patients who experienced a worsening after a response under conventional HLH treatment, the followings will be summarized
  - Time to Worsening defined as (date of worsening –date of response to previous treatment+1)
- For patients who experienced a Reactivation after a response under conventional HLH treatment, the followings will be summarized
  - Time to Reactivation defined as (date of Reactivation – date of response to previous treatment +1)
- Number of patients who experienced intolerance to conventional HLH treatment
- Number of patients who, having received previous HLH treatments, enter the study due to, respectively:
  - HLH reactivation after partial or complete response
  - HLH reactivation after incomplete response
  - No response/HLH worsening
  - Intolerance to treatment

Patients who had received previous treatments and entered the study for multiple reasons will be counted in each category of relevance.

- **Summary of medical history other than HLH disease**
  - Medical history will be summarized including the number (and percentage) of patients with at least one record of medical history, by SOC and PT. For patients with a disease history classed as 'Other' in the 'outside of HLH' electronic case report form (eCRF) page, the number and percentage of patients will be presented for each disease
- **Summary of prior medication, other than HLH treatments**
  - Number of patients by medication/procedures will be summarized.
- **Summary of HLH disease characteristics at Screening and Baseline**
  - Number of patients meeting each HLH criteria at screening and baseline
  - Number of patients presenting with CNS involvement at screening and baseline.
  - The above information will be summarized for 2nd Line All Treated and 2nd Line Evaluable Analysis Sets.

4.5.2 Summary of Other Screening/Baseline Characteristics

The values measured at Screening and at Baseline will be summarized for the following parameters using descriptive statistics, unless otherwise specified:

- Physical examination by body system (Normal, Abnormal, Missing)
- Vital signs

- Hematology and clinical chemistry parameters
- Cerebrospinal fluid (CSF) alterations

All Demographic, Screening and Baseline data will be provided in data listings.

4.6. Efficacy Evaluation

The evolution of clinical signs (fever, spleen and liver size) and laboratory parameters (hemoglobin, neutrophil and platelet counts, ferritin, fibrinogen, d-Dimer, triglycerides, AST, ALT, γGT, total bilirubin, LDH, CRP levels) characterizing HLH disease, will be presented using descriptive statistics at the following time points:

a. Baseline
b. SD14 (week 2)
c. SD28 (week 4)
d. SD42 (week 6)
e. EOT 04
f. EOT 04/05

The primary efficacy analysis will be the analysis performed on the primary efficacy endpoint in the pivotal cohort, i.e., 2nd line All Treated Analysis Set. This analysis will be conducted in the 2nd line Evaluable Analysis Set as supportive evidence for efficacy, and on the All Treated population. Secondary efficacy endpoints will be evaluated in a similar way.

Statistical significance in terms of p-values will only be obtained for the primary endpoint in the analysis sets as outlined above. The secondary endpoints will be viewed as supportive for the primary endpoint analyses and as such, no formal testing of endpoints will be undertaken.

4.6.1 Primary Efficacy Endpoint

4.6.1.1 Primary Efficacy Analysis

The primary efficacy endpoint is Overall Response, i.e., achievement of either Complete or Partial Response or HLH Improvement (as defined in Table 2-2), at EOT 04.

Data collected at the EOT of the NI 0501-04 study will be analyzed. EOT 04 is defined as 3 days after the last infusion in Study NI 0501-04, with a -3/+5 day time window allowed to minimize the number of missing parameters, provided that a given assessment is performed before conditioning.

The primary analysis of the primary endpoint will utilize an exact binomial test to evaluate the null hypothesis that the Overall Response Rate is at most 40%.

This test will be undertaken at the one-sided 0.025 level. The 2-sided exact 95% confidence interval will be provided. The 2-sided 95% confidence interval calculated via the Wilson score method will also be provided as supportive.

Overall Response Rate will be also presented for 1st line patients as well as by continent (US vs EU) with the corresponding two-sided 95% confidence limits, calculated via the exact and Wilson score methods.

Imputation for Overall Response:

For defining the primary efficacy endpoint (Overall Response), values of some parameters may be missing, thus impacting the response assessment. Imputation rules for assessing disease response at EOT and throughout the study have been pre-defined as follows:

Response at EOT

- Missing data at EOT (i.e. 3 days after the last infusion in Study NI 0501-04) for the parameters relevant to assessment of Overall Response will be imputed by the closest available value within -3 and +5 days before and after the EOT date respectively (provided upper window limit is before start of conditioning), e.g., EOT missing value at SD60 will be imputed by the closest assessment performed between SD57 and SD65 provided that this assessment was performed before conditioning. If assessments are available on the same number of days from EOT (one before and one after), then the assessment on the day after EOT will be selected provided that this assessment was performed before conditioning. For body temperature, in the event of repeated measures recorded on the same study day, the highest value will be selected.

- No imputation will be applied if no value is available within the -3/+5 time window. That parameter will be viewed as missing and complete response will not be assigned, even if all other parameters are normal.

- Values recorded at both scheduled and unscheduled visits will be considered.

Response throughout the study

- For secondary time to event analyses (e.g., duration of response, time to response), both scheduled and unscheduled visits will be used for assessment of response.

- If a parameter needed for the response assessment is missing, the midpoint approach will be used to estimate the missing value – i.e., the last observed value before the missing period will be averaged with the first value available after the missing period. Imputed values will be flagged in the listings. This rule will be applied to parameter values missing for a period of up to a maximum of 7 consecutive days. In case that, after imputation, data is still missing in any of the criteria considered for the definition of response, a Complete Response cannot be adjudicated.

- Since data relevant to CNS assessment has been collected at irregular time-points (based on individual patient conditions), the experts' assessment of CNS status will be carried-forward until the time-points of relevance for response analysis (e.g. a CNS improvement assessed at SD7 will be considered for ORR at week 2 if CNS assessment was not performed at week 2).

For descriptive statistics on the evolution over time of clinical signs and laboratory parameters characterizing HLH disease, the time windows listed in Table 4-1 will be applied. For patients completing the study before week 8 (i.e., between week 4 and week 8), values until the EOT time point will only be considered.

All data considered for the assessment of Overall Response will be provided in by-patient listings.

4.6.1.2 Sensitivity Analyses – Primary Endpoint

Sensitivity analyses will be performed on the primary efficacy endpoint using the above described exact binomial test and Wilson score methods for confidence intervals in the 2nd Line All Treated and All Treated Analysis Sets as follows:

- *Sensitivity Analysis 1:* Overall Response at EOT 04 with +/-3 days of imputation window for EOT visit
- *Sensitivity Analysis 2:* Overall Response at EOT 04 with patients who receive concomitant administration of other HLH treatments within the NI-0501-04 study considered as non-responders (i.e., Overall Response = 'No'), a -3/+5 days imputation window for EOT visit will be applied
- *Sensitivity Analysis 3:* Overall Response at EOT 04/05 (i.e., including continuation of NI-0501 treatment in the NI-0501-05 study, as relevant), with -3/+5 days of imputation windows for EOT visit
- *Sensitivity Analysis 4:* Overall Response at EOT 04 excluding patients who receive concomitant administration of other HLH treatments within the NI-0501-04, a -3/+5 days imputation window for EOT visit will be applied

Taking into account the potential impact of fluctuations in HLH parameters within the EOT assessment window and considerations from regulatory authorities review, the primary analysis and above sensitivity analyses will be repeated using the median value of each parameter within the 8-day window. These analyses will provide an opportunity to assign response status based on a robust estimate of the most typical value within the assessment window, providing further supportive data on overall efficacy.

4.6.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints include:

- **Time to First Response:** Time to first maintained response is defined as elapse time from date of first dose to first achievement of a response maintained for at least 4 day prior to or at EOT 04 (or EOT 04/05) Visit. Patients without an event will be censored at the EOT visit date or last assessment. Patients with no post-baseline assessments (assessment after the first infusion) will be censored at the first dose date.
- **Durability of First Response until EOT 04**: Maintenance of response for at least 4 days, achieved at any time during the study until EOT of the NI-0501-04 study. Durability of response is defined as the total elapsed time from achievement of first Overall Response to first loss of response, pending the duration is at least 4 days. If the first observed response does not last for at least 4 days, then the next response period will be considered. Similarly, loss of response should persist for at least 4 days, otherwise the next loss of response period will be considered. Patients who are in response at EOT 04 will be censored at that date. Patients who do not achieve at least HLH improvement once between the date of first dose and EOT 04 will be excluded from the analysis.

  Above analysis will also be performed by continent.

Swimmer plots will be presented to show response progression and durability by patient.

- **Cumulative duration of response NI-0501-04/05:** Total time in response from the first achievement of an Overall Response until HSCT conditioning (or EOT 04/05 if the patient did not have HSCT performed), considering that for patients who achieve a response, lose that response and then achieve it subsequently, the total time in response will be calculated by adding together these separate periods in response. Patients who are in response at the time of conditioning start will be censored at that date. Patients who do not achieve response at least once between the date of first dose and the time of conditioning start are excluded from the analysis.

  Above analysis will also be performed by continent.

  The start date of conditioning will be derived based on the start date of the first medication administered for HSCT conditioning, as described in Appendix 0.

- **Overall Response at Week 2 of treatment:** defined according to the same response criteria as used in the primary efficacy analysis and the visit window described in Table 4-1.
- **Time to HSCT:** Time to HSCT is defined as the time from the date of first dose to the date of HSCT. Patients who did not have HSCT are censored at the date of death or date of last contact (if no date of death). Median follow up time will be summarized for patients overall, patients proceed to HSCT, and patient that are censored.
- **Binary secondary endpoints**:
  - **Number of patients able to reduce glucocorticoids** by 50% or more of baseline dose at EOT 04 and at EOT 04/05 (see Appendix 7.2).
  - **Number of patients able to proceed to HSCT (**when deemed indicated**)**
  - **Post HSCT engraftment rates:** based on the number of patients experiencing primary or secondary graft failure, as reported in the AE module of eCRF, Appendix 7.5.
  - **Post HSCT acute and chronic GVHD incidence:** based on occurrence of GVHD, as reported in the AE module of eCRF, Appendix 7.5.
  - **Post HSCT HLH relapse rates:** as reported in the AE module of the eCRF, Appendix 7.5.

  Note: information on HSCT and post-HSCT outcome measures is collected in either the NI-0501-04 or NI-0501-05 study.

- **Assessment of Response by the Investigator at the per-protocol time points (i.e., SD15, SD27 and EOT)** Response frequencies will be presented by time point. Investigator assessments will also be listed.
- **Survival**
    - Overall Survival, defined as the time from the date of first dose to the date of death. Patients without an event will be censored at last assessment date in either the NI-0501-04 or NI-0501-05 study.

      Overall Survival will be presented by continent as well (US vs EU).
    - Overall Survival with inclusion of transplant as a time-dependent covariate to model the effect of receiving HSCT using Cox Regression.
    - Overall Survival post Week 2 by responder status Kaplan Meier estimates and plots will be presented by responder status at Week 2. Only patients who survive to Week 2 will be included in this analysis.

      Survival pre HSCT, defined as the time from the date of first dose to the date of death. Patients who receive an HSCT will be censored at that date. Patients who did not receive HSCT will be censored at last of contact date.

      This analysis will be presented by continent as well (US vs EU).
    - Survival post HSCT, defined as the time from the date of HSCT to the date of death. Patients without an event will be censored at last assessment date in either the NI-0501-04 or NI-0501-05 study. Patients who do not proceed to HSCT will be excluded from this analysis.

      This analysis will be presented by continent as well (US vs EU).
    - Event Free Survival: time from HSCT to any event (graft failure, HLH reactivation or death of any cause). If a patient did not have an event they will be censored at last observation. Graft Failures and HLH reactivation defining AE terms are listed in Appendix 7.5.

For all time to event analyses, such as Time to Response, Durability of Response, Survival, Kaplan Meier curves will be provided together with number of patients at risk through time and median time to event (where available) together with an associated two-sided 95% confidence interval. Kaplan-Meier estimates and median survival times are calculated with the PROC LIFETEST procedure in SAS.

For the binary secondary endpoints, including number of patients who reduce glucocorticoids by 50% or more, number of patients able to proceed to HSCT, Post HSCT engraftment rates, Post HSCT acute and chronic GVHD incidence, and Post HSCT HLH relapse rates, the number and proportion of patients will be provided with two-sided 95% confidence intervals.

Imputation rules for missing values described in Section 4.6.1.1 will be applied.

4.6.3 Exploratory Analysis

- Best overall response rate and time to best overall response up to EOT 04 and up to EOT 04/05
- Overall response at start of conditioning for patients who proceeded to HSCT

4.7. Pharmacokinetic/Pharmacodynamic Evaluations

Pharmacokinetic and PK/PD analyses will be provided in a separate document.

For the purpose of the present SAP, the evolution of biomarkers (Total IFNγ, CXCL9, CXC10, sIL2R) will be presented using descriptive statistics at the following time-points (including windowing as described before):

a. Baseline
b. SD14 (week 2)
c. SD28 (week 4)
d. SD42 (week 6)
e. EOT 04
f. EOT 04/05

4.8. Safety Analyses

Safety analyses will be conducted using the All treated Analysis Set as well as the 2nd Line and 1st Line All treated Analysis Set, unless otherwise stated. Data of patients participating to study NI-0501-04 and continuing in study NI-0401-05 will be pooled for the Safety analyses.

Of note, safety analysis for infections and SAEs will be also conducted for the 1st line patients separately for the preconditioning period.

Evaluation of NI-0501 tolerability and safety will be based on the following parameters:

- Adverse events analysis will be performed on AE variables defined in Table 4-3. Treatment-emergent Adverse Events (TEAEs) will be considered for the analysis, except for the overview table where all on-study AEs will be summarized
- Laboratory parameters:
  - Complete blood count (CBC), lymphocytes subsets (for specified study days)
  - Coagulation tests (activated partial thromboplastin [APT], prothrombin time [PT]), d-Dimer and fibrinogen
  - Biochemistry: glucose and electrolytes, ferritin, C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γGT, LDH, triglycerides, bilirubin, albumin, creatinine and urea
  - Urinalysis
  - CSF data
- Vital signs: temperature, heart and respiratory rate, blood pressure, oxygen saturation

- Physical examination:
  - prior to infusion at pre-defined visits including Skin aspect/appearance, Jaundice, Purpura, Oedema, ENT examination, Lymphadenopathies, Liver, Spleen, Respiratory examination, Cardiovascular examination, Neurological examination, Abdominal examination. In addition, during and after infusion (upon to 24 h) change in skin coloration, rash and sweating
- Immunogenicity: development of anti-NI-0501antibodies

Safety analyses will be performed separately for the pre- and post-conditioning time periods and by the line of treatment (unless otherwise specified).

Data of patients who have not undergone HSCT will be analyzed in the pre-conditioning period.

4.8.1 Treatment Compliance and Exposure

The definitions of drug administration variables are provided in Table 4-2. Descriptive statistics of these variables will be provided.

Table 4-2: Definition of Drug Administration Variables

Statistics for Exposure (dosing)	**NI-0501-04**
Duration of dosing (days and weeks) in NI 0501-04	Last infusion date in NI-0504-04 - first infusion date+1 (days) (Last infusion date in NI-0504-04 - first infusion date+1)/7 (weeks)
Overall duration of dosing (days and weeks)	Last infusion date in NI-0504-04/NI-0501-05 - first infusion date+1 (Days) (Last infusion date in NI-0504-04/NI-0501-05 - first infusion date+1)/7 (weeks)
Cumulative dose per kg in NI-0501-04 study	Sum of total dose per kg administered from first infusion date until last infusion date in the NI-0501-04 study.
Overall cumulative dose per kg	Sum of total dose per kg administered from first infusion date until last NI-0501 infusion date in either NI-0501-04 or NI-0501-05 study.
Average dose frequency (in days) in NI-0501-04	Duration of dosing* (days) in NI-0501-04 / Total number of infusions in NI 0501-04
Average dose frequency (in days) in NI-0501-04/05	Duration of dosing(days) / Total number of infusions in NI-0501-04 and in NI-0501-05
Average dose (mg/kg) per day in NI-0501-04	Cumulative dose (mg/kg) in NI-0501-04/ duration of dosing in NI-0501-04 (days)
Average dose (mg/kg) per day in NI-0501-04/05	Cumulative dose (mg/kg) in NI-0501-04/05 / overall duration of dosing in in NI-0501-04/05

** Duration of dosing= treatment end date -treatment start date +1.*

4.8.2 Adverse Events

Adverse event analysis will be performed on AE variables defined in Table 4-3. Treatment-emergent AEs (TEAEs) will be considered for the analysis, except for the overview table where all on-study AEs will be summarized.

Table 4-3: Definition of Adverse Events

Variable	Definition
Adverse Events (AEs) (On-study)	All recorded Adverse events (collection of AEs starts after ICF signature)
Treatment-Emergent Adverse Events (TEAEs)	Any adverse events (serious and non-serious) with an onset after the start of first infusion
Pre-conditioning TEAEs	Any adverse events (serious and non-serious) with an onset date before the start date of conditioning for HSCT*
Post-conditioning TEAEs	Any adverse events (serious and non-serious) with an onset date on or after the start date of conditioning for HSCT*
TEAEs related to the study drug	Any adverse events with "Relationship to Study Drug" of "Related"
Treatment-Emergent Serious Adverse Events (TESAEs)	Any adverse events with an onset after the start of first infusion with "serious" of "Yes"
TEAEs leading to study drug withdrawal	Any adverse events with an "Action taken related to study drug" of "Dosing stopped"
TEAEs with fatal outcome	Any adverse events with an "Outcome of AE" of "Fatal"
TEAEs with Infections	Adverse events from the SOC Infections and infestations
Suspected Infusion Related reactions (IRR)	Adverse events reported within 24h after start of infusion excluding the following SOCs: "Infections and infestations" "Congenital familial and genetic disorders," "Neoplasms benign, malignant and unspecified (incl. cysts and polyps)," "Product issues," "Social circumstances" and "Surgical and medical procedures." If the onset time of the AE or the start time of the infusion is missing, then AE with an onset date equal to an infusion date or infusion date +1 will be considered as potential IRR.
Confirmed IRR	Suspected IRR assessed as related to NI-0501 either by the investigator or the sponsor

* Note: The start date of conditioning will be derived based on the start date of the first medication administered for HSCT conditioning, as described in Appendix 0.

The TEAE tables will summarize the incidence of TEAEs and the number of patients with at least one given TEAE (sorted in descending order of the total frequency count) unless otherwise indicated. A patient with more than one occurrence of the same TEAE will be counted only once in the total of those experiencing TEAEs. For the overall summary tables, information presented by severity (mild, moderate, severe) will be presented by the worst severity per patient. Any AEs with a missing severity will be reported in the "missing" category grade.

The following TEAEs will be summarized in tables:

- TEAEs by preferred term (PT), separately for Pre-conditioning and Post-conditioning

- TEAE by system organ class (SOC), PT and Severity, separately for Pre-conditioning and Post-conditioning
- TEAEs by SOC and PT, separately for Pre-conditioning and Post-conditioning. Number of Severe TEAE and patients with severe TEAE will also be presented in the same table
- Serious TEAEs by SOC and PT, separately for Pre-conditioning and Post-conditioning
- Serious Pre-conditioning TEAEs by SOC and PT, separately for first line and second line patients
- Serious and Severe Non-Serious TEAE by dose group until EOT NI-0501-04/05
- Serious and Severe Non-Serious TEAE by treatment duration
- TEAE related to study drug by SOC and PT, separately for Pre-conditioning and Post-conditioning
- TEAE related to infections by SOC and PT, separately for Pre-conditioning and Post-conditioning
- Pre-conditioning TEAE related to infections by SOC and PT, separately for first line and second line patients
- TEAE related to infections by pathogen, separately for Pre-conditioning and Post-conditioning
- Pre-conditioning TEAE related to infections by pathogen, separately for first line and second line patients
- TEAE related to infections by dose group
- TEAE related to infections by treatment duration
- TEAE related to suspected IRR by PT (decreasing order of frequency)
- TEAE related to suspected IRR by PT and severity
- TEAE related to confirmed IRR by PT (decreasing order of frequency)
- TEAE related to confirmed IRR by PT and severity
- TEAE related to confirmed IRR by dose group (for all confirmed IRRs and IRRs of SOC skin and subcutaneous disorders)

An overview table will be produced and present the number/percentage of patients and the AE incidence including all TEAEs listed above, all AEs, TEAEs leading to a fatal outcome, TEAE by maximum severity and TEAEs leading to withdrawal separately for Pre-conditioning and Post-conditioning. An overview table will be also produced in 1st and 2nd line patients separately for TEAEs related to infections and Serious TEAEs for the preconditioning period.

For incidence of TEAEs that are summarized by SOC and PT, number and percentage of patients with at least one TEAE and number of TEAE will be presented

Serious TEAEs and severe non-serious TEAEs will be presented by duration of treatment to evaluate any potential impact of NI-0501. The proportion of patients (%) with at least one event will be presented by 2 week-treatment periods until end of treatment (EOT 04/05) or HSCT conditioning whichever comes first. In the analysis, patients will be included for any specified treatment period only if being treated with NI-0501 and alive up to and including the treatment period, and this will be used as the denominator for the percentage.

For serious TEAEs and severe non-serious TEAEs, the proportion of events relative to the number of infusions will be presented by dose (≤1 mg/kg, 1 mg/kg - ≤3 mg/kg, 3 mg/kg - ≤6

mg/kg, >6 mg/kg). As patients were allowed to use different doses during the study, AEs will be grouped by the dose received at the time or before of the event.

By-patient listings will be provided separately for pre and post conditioning for the following:

- All serious AEs (SAEs)
- All TEAEs leading to study drug discontinuation
- All TEAEs leading to a fatal outcome
- All TEAE with infection
- Confirmed or suspected IRRs

4.8.3 Treatment-Emergent Adverse Events with Infections

All TEAEs part of the SOC "infection and infestation" will be considered for this analysis.

Infections will be analyzed separately pre and post conditioning. An additional analysis will be performed on 1st and 2nd line patients for the preconditioning period.

The number of TEAEs with infections overall will be presented by duration of treatment as follows: the number (%) of patients with at least one event will be presented by 2 weeks treatment periods until EOT 04/05 or HSCT conditioning whichever comes first. In the analysis, patients will be included for any specified treatment period only if being treated with NI-0501 and alive up to and including the treatment period, and this will be used as the denominator for the percentage Adverse Events Related to Infusion-Related Reactions (IRRs).

Due to the frequency of NI-0501 infusions, assessment of IRRs using the normally temporal relationship would not result in an appropriate evaluation. Therefore, IRR analysis will follow a 2-step approach:

- Any TEAE from the predefined SOC (see Table 4-3) with an onset within 24h after start of an infusion will be considered as suspected IRR and presented by PT with incidence, severity and action taken.
- Suspected IRRs assessed as related to NI-0501 by the investigator or the sponsor will be considered as confirmed IRRs and presented by PT with incidence, severity and action taken

Confirmed IRRs will also be presented by dose (≤1 mg/kg, 1 mg/kg - ≤3 mg/kg, 3 mg/kg - ≤6 mg/kg, > 6mg/kg) with the frequency and percentage of events reported relative to the number of infusions performed at a given dose. As dose modifications may occur during study, the dose group in which an event will be classified will be determined depending on the dose the subject was receiving at the onset of the particular event. These events will be presented until EOT 04/05.

Adverse events related to documented infections (i.e., when a pathogen has been identified) will be presented in a data listing.

4.8.4 Laboratory Data

4.8.5.1 Hematology and Clinical Chemistry

Clinical laboratory values will be expressed using conventional units. Separate summary tables will be provided for data collected during the treatment period and the follow-up period. Details of visits to be presented are provided in Section 4.2.10.

Selected hematology (WBC, hemoglobin, neutrophils and lymphocytes, platelets), coagulation (PT, d-dimers, fibrinogen), and clinical chemistry parameters (ferritin, ALT, AST, total bilirubin, gGT, LDH, urea, creatinine, albumin, CRP) will be presented by visits as specified in Section 4.2.10. In addition, creatinine clearance will be derived from serum creatinine using the Schwartz equation:

CrCl (ml/min/1.73m2) = [length (cm) x k] / Scr (mg/dL)

With k = 0.45 for infants 1 to 52 weeks old
k = 0.55 for children 1 to 13 years old
k = 0.55 for adolescent females 13-18 years old
k = 0.7 for adolescent males 13-18 years old

In the event of repeat values on the same day, the earliest non-missing post 4 am value per study day will be used.

Shift tables will present changes from baseline to worst on-treatment and EOT 04/05 values relative to lab normal ranges, except for ALT, AST, gGT, and total bilirubin for which a medically relevant multiple of ULN will be presented. For ALT, AST, and gGT medically relevant multiples are 2.5xULN, for total bilirubin 1.5xULN. Missing categories will be included.

Box plots for the selected lab parameters defined above will be presented graphically for baseline, EOT 04/05 and according to the maximum (minimum) value during treatment based on:

- Absolute value
- Change from baseline (percentage or ULN multiples, as appropriate)

In addition, scatterplots by patient over time until EOT 04/05 will be presented for the selected lab parameters. In these plots, all values collected will be included (both scheduled and unscheduled visits).

For chemistry parameters, lab abnormalities and treatment-emergent lab abnormalities by CTCAE or WHO toxicity grading scale will be summarized separately for Pre-conditioning and Post-conditioning period.

All laboratory data will be provided in data listings. All laboratory data will be listed by patient number and visit and flagged according to pre/post-conditioning. Values outside laboratory reference ranges (as above described) will also be flagged.

4.8.5.2 Urinalysis

All urinalysis data will be provided in data listings.

4.8.5.3 Cerebrospinal Fluid Data

The following information has been collected during the study with regard to CSF analysis (as available in the lab reports):

- Appearance
- Color
- Protein
- Glucose
- RBC
- WBC
- Neopterin
- Lactate

All CSF data will be provided in data listings.

4.8.6 Vital Signs and Physical Examinations

4.8.6.1 Vital Signs

Summary statistics for vital signs parameters including body temperature (Celsius), systolic and diastolic blood pressure (mmHg), heart rate (beats/min), respiratory rate (breaths/min) and oxygen saturation (%) will be presented.

The actual values and change from baseline will be summarized by visits defined as follows: Screening, Baseline, SD0 (measurements surrounding first infusion), SD3, SD6, SD9, SD12, SD15 followed SD21, SD28, SD35 (etc, weekly) until EOT 04/05 (with visit windows of +/-3 days). In the event of repeat values, the earliest non-missing post 4 am value will be used.

At first infusion, vital signs have been assessed at the following time points:

- prior to each infusion
- during infusion
  - every 5 min during the first 15 min of the infusion and then
  - every 10 min until completion
- After the infusion
  - every hour for the first 4 hours after the infusion, then
  - every 2 hours for the next 12 hours
  - every 4 hours until completion of the 24 hour-monitoring
  - every 8 hours until the next infusion

For the first infusion, data recorded pre, during and post-infusion for selected vital sign parameters (as defined above) will be summarized by time point and will present descriptive statistics of:

- Actual value
- Change from pre-infusion measure

At other infusion visits, vital signs have been assessed at the following time points:

- prior to each infusion

- every 15 min during infusion
- every 4 hours after the infusion until completion of the 24 hour-monitoring
- every 8 hours until the next infusion (these evaluations were to be performed for all infusion visits in Treatment period 1)

For these other infusions, data recorded pre, during and post-infusion for the above defined vital signs will be summarized by infusion and by time point for all infusion in the first 2 weeks of treatment, and for the infusions where a dose increase have occurred, with descriptive statistics of:

- Actual value
- Change from pre-infusion measure

Vital signs will be listed by patient number and visit. Values outside the normal range (±10%) will be flagged in the individual data listings. For body temperature, values ≥37.5 °C will be flagged.

Reference ranges are age-specific and are listed in Table 4-4.

Table 4-4: Reference Ranges for Vital Signs

Age[1]	Heart Rate at Rest	Respiratory Rate at Rest	Systolic Blood Pressure	Diastolic Blood Pressure
0-3 months	100-150	35-55	65-85	45-55
>3-6 months	90-120	30-45	70-90	50-65
>6-12 months	80-120	25-40	80-100	55-65
>1-3 years	70-110	20-30	90-105	55-70
>3-6 years	65-110	20-25	95-100	60-75
>6-12 years	60-95	14-22	100-120	60-75
Over age 12	55-90	12-22	100-135	65-85
Over age 17	50-90	12-20	100-140	65-90

[1] *The age at visit date defined as [(visit date – date of birth+1)/365.25] should be used for selecting the normal ranges.*

Adapted from Charts for heart rate, respirations, and blood pressure taken from Robert M. Kliegman, et al., editors, Nelson Textbook of Pediatrics, 18th edition (Philadelphia: Saunders Elsevier, 2007), 389 and from paediatric life support guidelines (European Resuscitation Council 2010).

4.8.6.2 Physical Examination

Physical examination parameters include Skin aspect/appearance, Jaundice, Purpura, Oedema, ENT examination, Lymphadenopathies, Liver, Spleen, Respiratory examination, Cardiovascular examination, Neurological examination, Abdominal examination.

These physical examinations parameters will be summarized by visit indicating the number and percentage of patients presenting abnormality as follows: Screening, Baseline, SD0, SD3, SD6, SD9, SD12, SD15 followed by SD21, SD28, SD35 (etc., weekly) until EOT 04/05 (with visit windows of +/-3 days). In the event of repeat values on the same day, the earliest non-missing post 4 am value will be used.

At first infusion, Rash, Skin Coloration, and Sweating have been evaluated at the following time points:

- prior to each infusion
- every 5 min during the first 15 min of the infusion and then
- every 10 min until completion
- every hour for the first 4 hours after the infusion, then
- every 2 hours for the next 12 hours
- every 4 hours until completion of the 24 hour-monitoring

At other infusion, Rash, Skin Coloration, and Sweating have been evaluated at the following time points:

- prior to each infusion
- every 15 min during infusion
- every 4 hours after the infusion until completion of the 24 hour-monitoring

All abnormalities related to Rash, Skin Coloration, and Sweating assessed as medically relevant were to be recorded as AEs and will be analyzed in the IRR section.

A by-patient listing will be provided for:

- All physical examination data collected prior to each infusion including screening visit, On-treatment visit, unscheduled visit, and follow-up visit with a flag indicating post-transplant visits
- All Rash, Skin Coloration, and Sweating data collected during and after each infusion

4.8.6.3 Immunogenicity

The number and the percentage of patients with Anti-Drug Antibodies (ADAs) present will be summarized by assessment point. A separate immunogenicity report will be prepared and will be provided as Appendix to the CSR.

4.8.7 Electrocardiogram

ECG central reading will be performed by a specialized CRO (Cardiabase Europe, part of Banook Group) and a separate report will be appended to the CSR.

4.8.8 Imaging Test Results

Abnormalities from abdominal US, Chest X-ray and Brain MRI assessed as medically relevant were to be recorded as AEs and will be presented in AE tables.

Longitudinal spleen measures (as recorded at abdominal US) will be summarized at baseline and EOT (as available) in terms of frequency of Normal/Abnormal. As reference for normality, the suggested by-age Upper Limit of Normal is derived for each individual measure by the publications of Rosenberg et al., AJR 157:119, 1991.

A by-patient listing will be provided.

4.8.9 Hematopoietic Stem Cell Transplantation (HSCT)

For patients undergoing HSCT, either in the NI-0501-04 or in the NI-0501-05 studies, data relevant to the transplantation procedure will be summarized or listed with regards to:

- donor type
- degree of match
- stem cell source
- graft manipulation (if any)
- time to HSCT

4.8.10 Systematic Search for Infections

Abnormal results of TB search as well as adenovirus, CMV and EBV PCR assessed as medically relevant were to be recorded as AEs and will be presented in AE tables.

A by-patient listing will be provided.

4.8.11 Concomitant Medications

A prior medication/therapy is defined as any medication/therapy recorded in the NI-0501-04 study with end date prior to the first NI-0501 infusion date or recorded as ongoing=No, if end date is unknown.

Concomitant medication are medications taken on or after the first dose of study drug (start date/time is on or after the first dose of study medication, end date/time is after the first dose of study drug), or recorded as Ongoing as “YES” if the end date is unknown.

Concomitant medications will be coded using the WHO Drug dictionary version 2019. For concomitant medication originally coded by WHO Drug dictionary version 2015, codes will be updated in case changes between the two versions have occurred.

Number of patients and the corresponding percentages will be provided for patients having at least received one the following selected concomitant medication: diuretics, anti-hypertensive agents, anti-microbial agents (anti-fungal, antiviral, antibacterial), anti-cholestatics, vasopressive agents, anti-convulsive agents, immune-suppressants, biologics, etoposide (as defined in Appendix 7.6 and Appendix 7.7).

Concomitant medication will be summarized as ongoing at first NI-0501 infusion, administered until conditioning, and administered after conditioning.

Dexamethasone administered during NI-0501 treatment will be summarized separately: descriptive statistics of daily dose (mg/m2) will be presented at the following time points (including windowing as described before):

a. Baseline
b. SD14 (week 2)
c. SD28 (week 4)
d. SD42 (week 6)
e. EOT 04
f. EOT 04/05

Calculation of dexamethasone daily dose is specified in Appendix 7.2.

Drugs administered for HSCT preparation (conditioning) and GvHD prophylaxis (listed in Appendix 0) will be summarized separately, by type of drug, dose and duration.

All concomitant medication will be included in by-patient data listings.

In addition, two separate listings will be provided for HSCT conditioning and GvHD prophylaxis concomitant medications.

4.8.12 Pregnancy

Complete listings and narratives of all pregnancies (if any) with available pregnancy outcome data including maternal and fetal medical outcomes will be provided.

5. CHANGES TO PLANNED ANALYSES

A summary of the changes with respect to analyses described in the study protocol is given below:

- Intent-to-treat (ITT) analysis set and Per-Protocol Analysis Set were proposed in the protocol; they are not considered applicable, given the open-label, single-arm design of the study, and are not used in the SAP. All efficacy analyses are undertaken on all treated analysis set and evaluable analysis set, and subgroup population as appropriate.
- Secondary efficacy endpoint: Overall Response at Week 2 and Investigator's response assessment have been also analyzed.
- Secondary efficacy endpoint: Time to response and duration of response have been analyzed with the following specification:
  - both a response and a loss of response have to be maintained for at least 4 days in order to be considered in the analyses.
- Secondary efficacy endpoint: Cumulative duration of response (from start of treatment until HSCT conditioning) has been added to protocol specified endpoints.
- Secondary efficacy endpoint: Time to HSCT has been added to protocol specified endpoints.
- Secondary efficacy endpoint: Survival at Week 8 (or EOT) and at the end of the NI-0501-04 study are not presented separately, since they are covered by the Kaplan-Meier analyses of Overall Survival and Survival pre-HSCT.
- Secondary efficacy endpoints: Event-free survival has been added to protocol specified endpoints.
- Secondary efficacy endpoints: Exploratory analysis of best overall response and time to best overall response up to EOT 04 and up to EOT 04/05 has been added to protocol specified endpoints.
- PK analysis is not covered by the SAP, since PK analysis is performed through modelling separately and reported in a separate population PK report.

6. REVISION HISTORY AND SUMMARY OF CHANGES

VERSION #	DATE	AUTHOR	DESCRIPTION
Final Draft 1.0	12-Oct-2015	[redacted]	Internal use only
Final Draft 2.0	02-Nov-2016		Final draft submitted to RAs (FDA, EMA)
Final 3.0	01-Aug-2017		First approved version
Final 4.0	27-Oct-2017		Updated approved version
Final 5.0	20-Sep-2019		Updated approved version

- ***From SAP 1.0 to SAP 2.0***

  SAP 1.0 was drafted with reference to the NI-0501-04 pilot protocol (US protocol version 4.0; EU protocol version 5.0), and was therefore replaced by SAP 2.0 when the protocol was amended to continue as a Phase 2/3 study.

  The main modifications affecting the plan for statistical analysis can be summarized as follows:

  a. Definition of the efficacy endpoints (primary and secondary) that were exploratory in the pilot protocol

  b. Revision of criteria for the definition of response to treatment

  c. Definition of the null hypothesis in the context of formal sample size estimation

  d. Change from Bayesian to frequentist statistics.

- ***From SAP 2.0 to SAP 3.0***

  SAP 2.0 has been modified while finalizing TLFs and following comments received from Regulatory Agencies as follows:

  a. Section 3.2 now specifies the categories of protocol deviations that will be listed and summarized

  b. Section 4.2.9 has been modified to allow consideration to be given to the record "ongoing Y/N" when dealing with missing end dates for medication

  c. Section 4.2.10 has been modified to allow for wider visit windows in order not to unnecessarily exclude patients from analyses. Definition of visit windowing for time to event analysis has also been added

  d. Section 4.4 now includes the definition of study completion for both study NI-0501-04 and NI-0501-05

  e. Section 4.6.4 has been modified to include analysis of primary efficacy endpoint in 1st line patients separately

f. Section 4.6.5 has been modified as follows:
   - Durability of Response until EOT 04 is now identified as the main analysis for this secondary efficacy endpoints. Other time-frames for the assessment of Durability of Response have been considered among the exploratory analyses (section 4.6.6)
   - Post-HSCT outcome measures are now specified among the secondary efficacy endpoints
   - Competing risk analysis has been added to assess mortality post-HSCT
   - Analysis of event-free survival has been added

g. Section 4.6.6 has been modified to include the additional exploratory analysis of Cumulative duration of response

h. Section 4.8 has been modified as follows:
   - safety analyses are to be performed separately for the pre-conditioning and post-conditioning time periods
   - presentation of TEAEs by treatment duration and by dose received has been added
   - analysis of infections is to be performed separately in 1st and 2nd line patients
   - adverse events related to Infusion-Related Reactions have been defined
   - analysis of laboratory data now includes creatinine clearance, derived using Schwartz equation

i. Appendix 7.1 has been added to detail specifications for derivation of Overall Response

j. Appendix 7.2 has been added to describe other derivations relevant to the analyses

k. Appendix 7.3 has been added to define attribution of lab normal ranges, when missing for that site or age range

- ***From SAP 3.0 to SAP 4.0***

  SAP 3.0 has been modified for final execution of TLFs. Analysis plan updates include additional detail in handling limitations in the data, but overall analysis plan remains the same. Changes throughout the document include minor editorial changes. The main modifications include the following:

  a. Clarification for time points and visit windows is added to Section 4.2.10

  b. Imputation rules for durability of response analysis is added in Section 4.6.2

  c. Additional detail has been added into Appendix 7.1 for overall response derivation. Appendix 0 is added

  d. In Appendix 7.2, additional information is added for identification of the 2nd line population

  e. Appendix 7.3 definition of attribution of lab normal ranges when missing for that site or age range has been removed

  f. Appendix 7.5 has been added to detail classification of acute and Chronic GVHD, HLH Reactivation, Graft Failure

  g. Appendix 7.6 and Appendix 7.7 have been added to detail classification of selected concomitant medications

  h. Appendix 0 has been added to detail classification of HSCT conditioning related concomitant medications

i. Appendix **Error! Reference source not found.** has been added to detail classification of adverse event by pathogen class

j. Appendix 7.9 has been added to detail classification of disease characteristics with Central Nervous System Involvement

k. Appendix 0 has been added to detail classification of adverse event Indicating Organ failure

- ***From SAP 4.0 to SAP 5.0***

  SAP 4.0 has been modified for final CSR, to further clarify details of the analysis (without updates to programming logic) and include additional analyses as a follow-up to regulatory information requests as part of EMA and FDA submission processes. The main modifications include the following:

  a. Clarification of exploratory analysis has been added to section 2.5.3 and section 4.6.3

  b. Additional subgroup analyses have been added to section 4.2.7

  c. Section 4.5.1 has been updated with additional information about NI-0501-05 study completion and discontinuation, additional information about previous HLH medication has been added

  d. Additional sensitivity analyses have been included for analysis of efficacy, including use of median values within the assessment window for the primary efficacy endpoint (Overall Response Rate at EOT 04).

  e. Additional analyses of laboratory results have been added to section 4.8.5

  f. Wording inconsistencies in the description of Partial response and HLH improvement derivation (Appendix 7.1) have been clarified.

  g. Conditioning meds derivation has been modified to include meds 16 days prior to HSCT instead of 18 days prior, in order to exclude medication possibly administered for reason other than conditioning.

  h. In Appendix 7.6, Etoposide and Immunosuppressants are not included in "Selected concomitant medication" since they are already analysed in the context of "Concomitant medication for HLH treatment".

  i. In Appendix 7.7, only biologics of interest have been selected for analysis to better focus on clinically relevant medication.

  j. Appendix 7.9 has been removed, as has the analysis of infection by pathogen class described in section 4.8.3 since categorization by pathogen class can only be done on a case-by-case review of several individual patient information. Numbering of subsequent appendices is thus updated.

  k. Appendix 7.11 has been added to provide clarification on derivation of daily dexamethasone dose based on dose frequency recorded in the database.

7. APPENDICES

7.1. Overall Response Detailed Derivation

The HLH parameters used to assess disease response according to the definition of response reported in Table 2-2 are described in Table 7-1. Details for deriving the overall response are described in Table 7-2.

Table 7-1: HLH Parameters for Assessment

HLH Parameter	Variables Used for Assessment	Definition of Normalization
Parameters that are measured for improvement		
1. Body temperature	SDTM.VS	Below 37.5°C in all measurements performed on that day
2. Spleen as assessed at physical examination	SDTM.PE	Reported as Normal (= 0 cm from costal margin)
3. Absolute Neutrophil Count and G-CSF administration	SDTM.LB; SDTM.CM where ATC code=L03AA	Absolute Neutrophil Count is equal/greater than 1.0 x 109/L, and no G-CSF has been administered in the previous 4 days
4. Platelet count and platelet transfusion	SDTM.LB; SDTM.PR where PRCAT = Transfusions	Platelet count is equal/greater than 100 x 10^9/L, and no platelet transfusion administered in the previous 4 days
5. Ferritin	SDTM.LB	Less than 2000 µg/L
6. Fibrinogen and D-dimers	SDTM.LB	Fibrinogen greater than 1.5 g/L OR D-dimers equal/less than 500 ug/L
7. CNS disease involvement, as assessed by medical team	SDTM.FA	Result at EOT04: CNS disease =’No’ or ‘Normalized’ means Normal and to be used for CR and PR assessment; CNS= ‘Improved’ is to be used for HLH improvement assessment
Parameters that are measured for worsening of disease		
HLH Parameter	**Variables Used for Assessment**	**Definition of Worsening**
sCD25 levels	SDTM.PD, where PARAMCD = ‘sIL2RA’	Percent Change from Baseline (for the last two sCD25 data points before assessment) is not > 200%
Ongoing AE which indicates presence of organ failure*	SDTM.AE, where organ failure is identified by preferred terms selected by the Novimmune medical team, listed in Appendix 7.10	No ongoing AE at time of response assessment

Table 7-2: Derivation of Overall Response

Response	Application of Criteria	Example
Complete Response	– All HLH parameters that were abnormal at baseline must be normalized – All other parameters that were normal at baseline must still meet the definition of normalized – No worsening of disease observed, as assessed by presence of ongoing selected AE or worsening of sCD25 levels	Subject started with 5 abnormal parameters. All 5 improved, and the remaining parameters continue to meet definition of normalized. There are no ongoing AEs that indicate organ failure.
Partial Response	– At least 3 of the HLH parameters that were abnormal at baseline must be normalized. The remaining may be the same or worse as baseline (unless 2 or more parameters no longer meet the definition of normality) Note: If 3 parameters were abnormal at baseline, only 2 need to be normalized. If 2 parameters were abnormal at baseline, only 1 needs to be normalized – No worsening of disease observed, as assessed by presence of ongoing selected AE or worsening of sCD25 levels	Subject started with 4 abnormal parameters. Three (3) of the parameters now meet definition of normalized. The fourth is still abnormal. No more than 1 parameter (normal at baseline) crosses the normality threshold . There are no ongoing AEs that indicate organ failure.

Response	Application of Criteria	Example
HLH Improvement	– At least 3 of the HLH parameters that were abnormal at baseline must be normalized or improved (i.e., >=50% change from baseline). The remaining may be the same or worse as baseline (unless 2 or more parameters no longer meet the definition of normality) Note: If 3 parameters were abnormal at baseline, only 2 need to be at least improved. If 2 parameters were abnormal at baseline, only 1 needs to be improved – No worsening of disease observed, as assessed by presence of ongoing selected AE or worsening of sCD25 levels Definition of 50% improvement from baseline: – Spleen size decreased by 50%, as recorded in cm from costal margin at physical examination. – Absolute Neutrophil Count increased by 50%, if G-CSF has not been administered in the previous 4 days – Platelet count increased by 50%, if no platelet transfusion has been administered in the previous 4 days – Ferritin decreased by 50% – Fibrinogen increased by 50% or D-Dimer decreased by 50% – CNS= 'Improved'	Subject started with 4 abnormal parameters. Two (2) of the parameters now meet definition of normalized. The third improved >50%, but is still abnormal. The fourth is still abnormal and did not improve >50%. No more than 1 parameter (normal at baseline) crosses the normality threshold. There are no ongoing AEs that indicate organ failure.
No Response	If a subject does not meet the criteria for at least HLH improvement, response is categorized as "No Response"	Subject meets the definition of partial response, but has worsening disease as identified by an ongoing AE indicating organ failure at time of assessment.

7.2. Derivation of Certain Analysis Variables

The following section provides instructions for the derivation needed for some variables required for analysis.

Identification of 2nd line patients

As per protocol definition, these are patients who have received conventional HLH therapy (as per site standard of care), e.g., any of the following alone or in combination (Etoposide, ATG, Alemtuzumab and Cyclosporine A) or glucocorticoids, namely Dexamethasone at 10 mg/m^2 for at least 7 days or methylprednisolone pulses for 3 consecutive days.

The following derivation will be done:

- Yes to Question: Received any medication to treat HLH since diagnosis [Medical History of HLH]

  and
- Medication that:
  - Contains text 'HLH 2004'.
    or
  - Medication recorded in eCRF with a start date prior to NI-0501 first infusion as follows: (priorities need to be followed as below list, check first condition first if not met then check second condition, if not met then the third and so on).

    - Etoposide [ATC04ID L01CB - Preferred code 00511901001], any dose, any duration
    - Antithymocyte immunoglobulin [ATC04ID L04AA - Preferred code 00575401001], any dose, any duration
    - Alemtuzumab [ATC04ID L04AA - Preferred code 01268601001], any dose, any duration
    - Cyclosporin A [ATC04ID L04AD - Preferred code 00549701001], any dose, any duration
    - Anakinra [ATC04ID L04AC - Preferred code 01345101001], any dose, any duration
    - Tocilizumab [ATC04ID L04AC - Preferred code 01759101001], any dose, any duration
    - Dexamethasone [ATC04ID H02AB - Preferred code 00016001001], dose ≥ 10mg/m2 for at least 7 days
    - Methylprednisolone [ATC04ID H02AB - Preferred code 00049601001], with a verbatim indicating "pulses" or "high dose", or any dose for more than 7 days or 30 mg/kg for 3 consecutive days

Dexamethasone daily dose

The dose of dexamethasone is to be expressed as mg/m^2/day in order to give homogeneous metrics across patients (see Appendix 7.11).

For patients with dose frequency captured as 'other' plus individual texts different from the ones described in Appendix 7.11, dosing regimen will be defined/specified and provided by Novimmune through an external spreadsheet 'Dexa daily dose file' based on review of final datasets.

Given the possibility of having dexamethasone doses recorded differently in eCRF, the following calculation will be performed:

- Dose of dexamethasone in mg * number of administrations per day =
  daily dose
- BSA Surface Area (BSA) [VS]
- Daily dose / BSA = dose in mg/m^2/day

Glucocorticoid tapering

For the reduction of glucorticoid dose by 50% or more of baseline dose, the use of glucocorticoid for systemic use (ATC04ID H02AB) during the NI-0501-04 study period will be obtained by the CM/PR domain of eCRF taking into consideration the followings:

- Dexamethasone (H02AB - PREFERRED CODE 00016001001) dose expressed as mg/m^2/day at
  - Baseline = SD-1 or closest day to SD0
  - The lowest dose received during the study and the corresponding SD,
  - The dose received at EOT 04

provided that:

- No other glucocorticoids have been received by oral or i.v. route on the SD of interest with reference to:
  - Methylprednisolone (H02AB - PREFERRED CODE 00049601001)
  - Prednisolone (H02AB - PREFERRED CODE 00016201001)
  - Prednisone (H02AB - PREFERRED CODE 00044701001)
  - Hydrocortisone (H02AB - Preferred code 00028601001, 00028603001,00028602001)

In that case, the dose of the other glucocorticoids has to be summed up, taking into consideration glucocorticoid conversion.

7.3. NI 0501-05 Study Synopsis

Title:	A Multicenter Study for the Long-term Follow-up of HLH Patients who Received Treatment with NI-0501, an Anti-interferon Gamma Monoclonal Antibody
Sponsor:	NovImmune SA, Switzerland
Study Type:	International, multicenter, long-term, follow-up study of HLH patients who have received NI-0501 in the context of the clinical development program for NI-0501 NI-0501-05 study is performed both in the US and in Europe according to twin protocols called NI-0501-05-P-IND #111015 and NI-0501-05-EudraCT #2012-005753-23, respectively
Study Population:	HLH patients who have received at least one dose of NI-0501. In the event that, at the end of NI-0501 induction treatment, an appropriate donor has not been identified or in case of the need to delay Hematopoietic Stem Cell Transplantation (HSCT) for reasons unrelated to the administration of NI-0501, patients may continue NI-0501 treatment in the context of this protocol upon request of the treating physician after having established a favourable benefit/risk from NI-0501 treatment.
Main Inclusion Criteria:	1. Having received at least one dose of NI-0501 during a previous NI-0501 study. 2. Having signed the Informed Consent by the patient or the patient's legal representative(s), as applicable, with the assent of patients who are legally capable of providing it.
Study Objectives:	- To monitor the long-term safety profile of patients who have received NI-0501 - To assess HLH patients' survival after NI-0501 treatment - To study the elimination profile of NI-0501 - To assess the immunogenicity of NI-0501
Study Drug:	No investigational medicinal product (IMP) will be administered during the course of this long-term follow-up study. However, in the event that, upon request of the treating physician, NI-0501 treatment needs to be prolonged beyond Week 8, patients will continue receiving NI-0501 in the context of this study. In this case NI-0501 will be managed as the IMP.
Investigating Sites:	All sites where patients have been recruited during the clinical development program of NI-0501.
Study Duration and	- The study will continue until data from month 12 post-

Study End Definition:	Transplantation are available - The study will end after the last patient has undergone the last visit
Concomitant Medications:	There is no restriction in the use of medications after discontinuation of NI-0501 treatment, except for live or attenuated live vaccinations that should be avoided as long as NI-0501 activity is present.
Study Parameters:	- Vital signs, including body temperature - Physical examination, including liver and spleen sizes - Laboratory parameters: complete blood count, coagulation tests including fibrinogen, inflammatory parameters (ferritin and CRP), glucose and electrolytes, liver (e.g., triglycerides, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, lactate dehydrogenase, bilirubin and alkaline phosphatases) and renal (e.g., creatinine, albumin and urea) function tests - Pharmacokinetics: circulating NI-0501 concentration • Pharmacodynamics: circulating IFNγ levels and exploratory markers of disease activity (e.g., sCD25) • Anti-drug antibodies (ADAs)

Study Endpoints:	- Safety:
	  - Incidence, intensity, possible relationship to NI-0501 and outcomes of Adverse Events (serious and non-serious)
	  - Evolution of vital signs, physical examination and laboratory values over time
	• Efficacy (when relevant):
	  - Clinical Response mostly based on HLH diagnostic criteria and existence of neurological symptomatology
	  - Survival before Transplantation, 100 days and one year after Transplantation
	• Pharmacokinetics: NI-0501 elimination profile (clearance and elimination half-life)
	• Pharmacodynamics: NI-0501 activity on IFNγ neutralization
	• Immunogenicity: presence of ADAs
	• Exploratory endpoints: e.g., additional markers of disease activity

7.4. Scheduled Assessments

Table 7-3: Schedule of Assessments – Screening & Treatment Period 1 – SD0 to SD15 (Weeks 1 and 2)

Assessments		Screening		Treatment Period 1 - SD0 to SD15 (wk 1 and 2)											
		Up to one week prior to first infusion	SD-1	Inf. 1			Inf. 2		Inf. 3		Inf. 4		Inf. 5		Inf. 6
				SD0	SD1	SD2	SD3	SD5	SD6	SD8	SD9	SD11	SD12	SD14	SD15
Hospitalisation			Starting from SD-1												After this time-point patients may be discharged
Dexamethasone			Starting from SD-1												
Prophilactic treatment, as described in Section 6.2			Starting from SD-1												
Infusion				x			x		x		x		x		x
Patient Information		x													
Clinical Assessment	Vital signs [1]		x	X (Pre, during, post)	x	x	X (Pre, during, post)	x	X (Pre, during, post)	x	X (Pre, during, post)	x	X (Pre, during, post)	x	X (Pre, during, post)
	Continuous cardiac monitoring / pulse oxymetry			X (Pre, during, post)			X (Pre, during, post)		X (Pre, during, post)		X (Pre, during, post)		X (Pre, during, post)		X (Pre, during, post)
	Physical Examination [2]	x	x	x (Pre)	x	x	x (Pre)	x	x (Pre)	x	x (Pre)	x	x (Pre)	x	x (Pre)
Procedure	ECG	x		x (Post)	only if clinically indicated										
Search for Infections	TB [3]	x						x					x (Pre)		
	Adenoviruses, EBV, CMV (viral load)	x						x					x (Pre)		
	HSV, HZV, HIV, HBV, HCV	x		In case of suspicion of infection											
	Atypical mycobacteria, *Histoplasma Capsulatum* , *Shigella* , *Salmonella Campylobacter* , *Leishmania*	x		In case of suspicion of infection											
Laboratory	CBC	x		x (Pre)	x (morning)	x (morning)	x (Pre)	x	x (Pre)	x		x		x	
	Lymphocyte subsets	x					x (Pre)		x (Pre)						
	Coagulation (aPTT, PT, D.-dimers), fibrinogen	x		x (Pre)	x	x	x (Pre)	x	x (Pre)	x		x		x	
	Biochemistry[4], triglycerides	x		x (Pre)	x	x	x (Pre)	x	x (Pre)	x		x		x	
	IgG level	x													
	Pregnancy test (if applicable)	x													
	Urinalysis[5]	x		x (Pre)[5]	x	x	x (Pre)	x	x (Pre)	x		x		x	
Imaging	3D abdominal US (spleen and liver size)	x													x
	Chest X-ray[6]	x													
	Brain MRI	In case of CNS symptoms occurrence													
Histopathology	Cerebrospinal Fluid (CSF) analysis if coagulation allows	x		Only if clinically indicated (to monitor evolution or to confirm occurrence of new CNS symptoms)											
PD/Exploratory (sCD25, IL-10, CXCL9, CXCL10, CXCL11), IFNγ (free IFNγ at SD0 predose, total IFNγ for all other timepoints)				x (Pre)	x	x	x (Pre)	x	x (Pre)	x	x (Pre)		x (Pre)		x (Pre)
PK (NI-0501 circulating concentration)				x (Pre - post infusion)	x	x	x (Pre - post infusion)	x	x (Pre - post infusion)	x	x (Pre - post infusion)		x (Pre - post infusion)		x (Pre - post infusion)
Immunogenicity (ADA)		x													

1: Vital signs: Temperature, heart rate, blood pressure, respiratory rate. Oxygen saturation is also recorded at SD-1 and pre-, during and after infusion on infusion days

2: Physical examination: : includes as a minimum: weight (at screening, at SD-1, and prior to each infusion), height (at screening only), and in particular at each visit, occurrence of skin rashes, jaundice, purpura, bleeding, edema, ascites, search for tonsillitis, lymphadenopathies, dyspnea, cough, spleen and liver size, and neurological examination

3: TB: search for tuberculosis mycobacteria: At screening: IGRA/PPD and PCR; after screening by PCR

4: Biochemistry= glucose, electrolytes, ferritin, CRP, AST, ALT, ALP, gGT, LDH, bilirubin, albumin, creatinin, urea

5: Urinalysis= glucose, blood, protoein, leukocytes, ketone, pH, gravity. On SD0 urinalysis needs to be performed if not done at screening

6: Chest X-ray: every 4 weeks, except if required more frequently in case of clinical suspicion of a pulmonary infection

Table 7-4: Schedule of Assessments - Treatment Period 2 – SD 16 to EOT (3 days after last NI-0501 infusion) (Weeks 3 to 8) & Follow-up Period

Assessments		Treatment Period 2 - Week 3-8 SD16 until EoT (3 days after last NI-0501 infusion)			Follow-Up Period[7]			Wk 4/ Study completion visit or Withdrawal (WD) visit	Unscheduled Visit (UV)[9]
		Infusion visit	Efficacy/Safety visit[6]	End of treatment visit					
		Infusion X	Efficacy/Safety visit X	3 days post last infusion (± 1 day)	Week 2	Week 3	Pre-conditioning visit[8]		
	Infusion	X							
Clinical Assessment	Vital signs [1]	X (Pre, during, post)	X	X	X	X	X	X	X
	Continuous cardiac monitoring/ pulse oxymetry	X (Pre, during, post)							
	Physical Examination [2]	X (Pre)	X	X	X	X	X	X	X
Procedure	ECG	only if clinically indicated		X	only if clinically indicated			X	
Infections	TB [3]		X (every 2 weeks)	X	X			X	
	Adenoviruses, EBV, CMV (viral load)		X (every 2 weeks)		X			X	
	Atypical mycobacteria, *Histoplasma Capsulatum* , *Shigella, Salmonella Campylobacter, Leishmania*	In case of suspicion of infection							
Laboratory	CBC		X	X	X	X	X	X	
	Coagulation, fibrinogen		X	X	X	X	X	X	
	Biochemistry[4], triglycerides		X	X	X	X	X	X	
	Urinalysis (glucose, blood, protein, leukocytes, ketone, pH, gravity)		X	X	X	X	X	X	
Imaging	3D abdominal ultrasound (spleen and liver size)		X (every 2 weeks)	X			X		
	Chest X-ray[5]		X (every 4 weeks)	X				X	
	Brain MRI	In case of CNS symptoms							
Histopathology	CSF analysis	only if clinically indicated							
PD/Exploratory[10]		X (pre)		X	X	X	X	X	
PK (NI-0501 circulating concentration)[10]		X (pre and post)		X	X	X	X	X	
Immunogenicity (ADA)				X				X	

1: Vital signs: Temperature, heart rate, blood pressure, respiratory rate. Oxygen saturation is also recorded pre-, during and after infusion on infusion days

2: Physical examination: : includes: as a minimum weight prior to each infusion, at each follow-up visit and each unscheduled visit; weight and height at the end of treatment visit and at Week 4/Study completion visit or Withdrawal (WD) visit and in particular at each visit, occurrence of skin rashes, jaundice, purpura, bleeding, edema, ascites, search for tonsillitis, lymphadenopathies, dyspnea, cough, spleen and liver size, and neurological examination

3: TB: search for tuberculosis mycobacteria by PCR

4: Biochemistry= glucose, electrolytes, ferritin, CRP, AST, ALT, ALP, gGT, LDH, bilirubin, albumin, creatinin, urea

5: Chest X-ray: every 4 weeks, except if required more frequently in case of clinical suspicion of a pulmonary infection. At EoT and follow-up visits, chest X-ray will not be performed unnecessarily if a recent exam is available

6: Efficacy/Safety visits: should occur every 6 days, with a time-window of ± 48 hours in order to combine, whenever possible, with NI-0501 infusion visits.

7: Pre-HSCT visit: if applicable, i.e. if transplant takes place during the 4-week follow-up period, appropriate schedule will be applied to combine a weekly follow-up visit with the pre-HSCT visit at the site.

8: Pre-conditioning visit: if applicable, i.e. if the patient starts conditioning during the 4-week follow-up period, the closer weekly follow-up visit will be combined, in order to allow collection of clinical and laboratory HLH parameters before administration of the conditioning drugs.

9: Unscheduled Visit: These assessments should be performed at minimum, but additional assessments may be added according to the clinical judgment of the Investigator.

10: PK/PD: Additional PK/PD samples may be required to better characterize the PK/PD profile and/or for further safety assessments. Number of additional samples taken will be based on body weight, patient characteristics and clinical status of the patient.

7.5. Adverse Events Classification by System Organ Class and Preferred Term for Acute and Chronic GVHD, HLH Reactivation, Graft Failure

System Organ Class Code	System Organ Class	Preferred Term Code	Preferred Term
Acute and Chronic GVHD			
10021428	Immune system disorders	10066264	Acute graft versus host disease in intestine
10021428	Immune system disorders	10066262	Acute graft versus host disease in skin
10021428	Immune system disorders	10018651	Graft versus host disease
10021428	Immune system disorders	10075160	Graft versus host disease in gastrointestinal tract
10021428	Immune system disorders	10064676	Graft versus host disease in liver
10021428	Immune system disorders	10064675	Graft versus host disease in skin
HLH Reactivation			
10018065	General disorders and administration site conditions	10010264	Condition Aggravated
Graft Failure			
10022117	Injury, poisoning and procedural complications	10060872	Transplant failure
10022117	Injury, poisoning and procedural complications	10068081	Engraft failure
10021428	Immune system disorders	10048396	Bone marrow transplant rejection
10022117	Injury, poisoning and procedural complications	10010162	Complications of bone marrow transplant

7.6. Selected Concomitant Medications by Anatomic Therapeutic Class (including Level if appropriate)

Selected Concomitant Medications	Anatomic Therapeutic Class (including Level if appropriate)
ANTICHOLESTATICS	ATC Level 2: A05
VASOPRESSIVE AGENTS	ATC Level 4: C01CA
DIURETICS	ATC Level 2: C03
ANTIHYPERTENSIVE AGENTS	ATC Level 2: C02, C07, C08, C09
ANTIBACTERIALS	ATC Level 2: J01
ANTIMYCOTICS	ATC Level 2: J02
ANTIMYCOBACTERIALS	ATC Level 3: J04A
ANTIVIRALS	ATC Level 2: J06
ANTICONVULSIVE AGENTS	ATC Level 2: N03

7.7. Biologics of Interest by Anatomic Therapeutic Class Level 4 and Preferred Text/Code

Preferred Code	Preferred Text	ATC Level 4
01268601001	ALEMTUZUMAB	L04AA
00575401001	ANTITHYMOCYTE IMMUNOGLOBULIN	L04AA
00575402001	ANTITHYMOCYTE IMMUNOGLOBULIN (RABBIT)	L04AA
02082701001	ANTILYMPHOCYTE IMMUNOGLOBULIN	L04AA
05699301001	ECULIZUMAB	L04AA
01514401001	RASBURICASE	V03AF
02600801001	PALIFERMIN	V03AF
01345101001	ANAKINRA	L04AC
01759101001	TOCILIZUMAB	L04AC
01411101001	BASILIXIMAB	L04AC
06441101001	FILGRASTIM	L03AA
01003401001	GRANULOCYTE COLONY STIMULATING FACTOR	L03AA
01490601001	ETANERCEPT	L04AB
01445601001	INFLIXIMAB	L04AB
07210601001	RUXOLITINIB	L01XE
01402501001	RITUXIMAB	L01XC

7.8. Concomitant Medications for HSCT Conditioning and GVHD Prophylaxis

HSCT CONDITIONING

Conditioning Agents to Consider [SDTM.CM]

ATC Level 4	Preferred Code	Preferred Text
L01AA	00021101001 00006401001	CYCLOPHOSPHAMIDE MELPHALAN
L01AB	00036801001 00418901001	BUSULFAN TREOSULFAN
L01AC	00053501001	THIOTEPA
L01BB	02122001001 01004601001 01004602001	CLOFARABINE FLUDARABINE FLUDARABINE PHOSPHATE
L01BC	00146201001	CYTARABINE
L01CB	00511901001 00511902001	ETOPOSIDE ETOPOSIDE PHOSPHATE
L01XC	01402501001	RITUXIMAB
L04AA	01268601001 00575401001 00575402001 02082701001	ALEMTUZUMAB ANTITHYMOCYTE IMMUNOGLOBULIN ANTITHYMOCYTE IMMUNOGLOBULIN (RABBIT) ANTILYMPHOCYTE IMMUNOGLOBULIN

Then:

For Patients with HSCT date [SDTM.PR]

- Med Start Date: >=HSCT date – 16 days (med should start not earlier than 16 days before transplant)

and

- Med End Date: <= HSCT date (med end date should be latest date of transplant)

For Patients without HSCT date [SDTM.PR]

- Med with Indication containing: Conditioning

Start of conditioning is the first start date of the selected drugs.

GVHD PROPHYLAXIS

Medication with Indication containing GVHD and Prophy [SDTM.CM].

7.9. Disease Indicating Central Nervous System (CNS) Involvement by Medical History/AE Preferred Term and Code

Preferred Term Code	Preferred Term
10029818	Nuclear magnetic resonance imaging brain abnormal
10059703	CSF test abnormal
10053805	CSF white blood cell count increased
10011522	CSF cell count increased
10035551	Pleocytosis
10012559	Developmental delay
10056832	Neurological examination abnormal
10047641	VIth nerve paralysis
10020745	Hyperreflexia
10041962	Status epilepticus
10039906	Seizure
10021118	Hypotonia
10002948	Aphasia
10029202	Nervous system disorder
10017577	Gait disturbance
10048334	Mobility decreased
10008096	Cerebral atrophy
10015037	Epilepsy
10071066	Posterior reversible encephalopathy syndrome
10022840	Intraventricular haemorrhage

7.10. Adverse Events Indicating Organ Failure by MedDRA Preferred Term and Code

Preferred Term Code	Preferred Term
10007554	Cardiac failure
10007556	Cardiac failure acute
10060953	Ventricular failure
10024119	Left ventricular failure
10063081	Acute left ventricular failure
10039163	Right ventricular failure
10063082	Acute right ventricular failure
10051093	Cardiopulmonary failure
10038695	Respiratory failure
10001053	Acute respiratory failure
10019663	Hepatic failure
10000804	Acute hepatic failure
10077305	Acute on chronic liver failure
10019845	Hepatorenal failure
10038435	Renal failure
10077361	Multiple organ dysfunction syndrome
10053159	Organ failure
10010264	Condition aggravated
10007554	Cardiac failure
10007556	Cardiac failure acute

7.11. Calculation of Dexamethasone daily dose

Dexamethasone daily dose will be calculated based on the recorded dose frequency as follows:

Dose frequency	**Total Daily dose= CMDOSE*factor**
QD	CMDOSE
BID	CMDOSE*2
TID	CMDOSE*3
QID	CMDOSE*4
OTHER - DAILY	CMDOSE
OTHER - EVERY 12 HOURS	CMDOSE*2
OTHER - EVERY 12 HRS	CMDOSE*2
OTHER - EVERY 12 HOURS	CMDOSE*2
OTHER - EVERY 12HRS	CMDOSE*2
OTHER - NOT KNOWN	NOT KNOWN
OTHER - HLH 2004	NOT KNOWN
OTHER - UNKNOWN	NOT KNOWN

If individualized specifications have been recorded (e.g. Other – 2.5 mg in the morning and 2 mg in the evening, Other – 3 x week, etc.), informarmation on the daily dose will be translated from natural language to standardized entries by Novimmune based on review of the single entries.