Statistical Analysis Plan for Interventional Studies (Early Phase)

Sponsor Name: Neuracle Science Co., LTD.

Protocol Number: NS101_P1_01

Protocol Title: A phase 1, randomized, placebo-controlled, double-blind, single ascending dose study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenous NS101 infusion in healthy volunteers

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Revision History

Version	Date (DD-Mmm-YYYY)	Document Owner	Revision Summary
1.0	26-Aug-2022	Sarah Lutkewitz	Initial version

Signature Approvals

I confirm that I have reviewed this document and agree with the content.

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Glossary of Abbreviations

Abbreviation	Description
ADA	anti-drug antibody
AE	adverse event
AUC	area under the concentration-time curve
AUC_{0-24}	area under the concentration-time curve, from time zero to 24-hours
AUC_{0-t}	area under the concentration-time curve from time zero until the last observed concentration
AUC_{0-inf}	area under the concentration-time curve from time zero to infinity
BLQ	below the lower limit of quantification
BMI	body mass index
CI	confidence interval
Cl	apparent body clearance
C_{max}	maximum observed concentration
CRF	case report form
CSF	cerebrospinal fluid
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
ECG	electrocardiogram
eCRF	electronic case report form
E_{max}	maximal observed effect
FIH	first-in-human
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IV	intravenous
K_{el}	elimination rate constant
LLOQ	lower limit of quantification
max	maximum
MedDRA®	Medical Dictionary for Regulatory Activities
min	minimum
n	number of observations
NAb	neutralizing antibody
OLS	ordinary least squares
PD	Pharmacodynamic(s)

Abbreviation	Description
PK	pharmacokinetic(s)
PT	preferred term
RTF	rich text format
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class
SOP	standard operating procedure
SRC	Safety Review Committee
S-STS	Sheehan Suicidality Tracking Scale
$T_{1/2\ el}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
TE_{max}	time of maximal observed effect
TLFs	tables, listings, and figures
T_{max}	time to reach the maximum observed post-dose concentration
V_z	apparent volume of distribution
WHOD	World Health Organization Drug Global Dictionary

1. Purpose

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables, and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives. Safety, tolerability, pharmacokinetic (PK), immunogenicity and pharmacodynamics (PD) analyses will all be described.

This SAP is based on the following documents:

- Protocol No. NS101_P1_01 Amendment III, dated 06-Apr-2022
- Electronic Case Report Form (eCRF) version 3.00, dated 17-May-2022
- Protocol Administrative Letter for Early Phase Studies dated 19-May-2022
- NS101_P1_01_Electronic CRF Completion Guidelines_Final 3.0

The plan may change due to unforeseen circumstances; any changes made after the plan has been finalized, will be documented. No revision to the SAP is required for changes which do not affect the statistical analysis methods, definitions, or rules defined in this document. If additional analyses are required to supplement the planned analyses described in the SAP, the changes and justification for the changes will be outlined in the associated clinical study report (CSR). No change will be made without prior approval of the Sponsor.

When applicable, all methodologies and related processes will be conducted according to Syneos Health Standard Operating Procedures (SOPs) as appropriate. Shells for all statistical tables, listings, and figures referred to in this SAP will be displayed in a separate document.

1.1 Responsibilities

Syneos Health will perform the statistical analyses and are responsible for the production and quality control of all safety, PK, immunogenicity and PD analysis and tables, listings, figures (TLFs).

1.2 Timings of Analyses

Interim Analysis:

Interim analysis of safety and PK will be performed for each dose level using available blinded serum concentrations data (up to 48 hours post-start infusion) will be reviewed at Safety Review Committee (SRC) meeting prior to dose escalation.

Final Analysis:

The final analyses of safety, PK, immunogenicity, and PD will be completed after subjects complete the final study visit or terminate early from the study.

2. Study Objectives

- Primary objectives:
  - To assess the safety and tolerability of NS101 following intravenous (IV) administration of single ascending doses in healthy subjects
- Secondary objectives:
  - To characterize the PK profile of NS101 in serum and NS101 concentrations in cerebrospinal fluid (CSF) following single IV infusion doses in healthy subjects.
  - To characterize the PD profile of NS101 through FAM19A5 in plasma and FAM19A5 concentrations in CSF following single IV infusion doses in healthy subjects.
  - To evaluate the immunogenicity profile of NS101.

3. Study Description

This study is a single center Phase I, first-in-human (FIH), single ascending dose (SAD), double bind study to evaluate the safety, tolerability, PK, PD, and immunogenicity of NS101 in healthy volunteers and aid in the selection of optimal doses and dosing regimens for future studies. The study consists of up to 8 cohorts. The cohorts will be dosed sequentially.

3.1 Subject Selection

Up to 80 healthy, non-smoking, male volunteers aged 18 to 55 years, with body mass index (BMI) >18.5 and <30.0 kg/m^2 and body weight ≥50.0 kg, will be enrolled in this study.

3.2 Determination of Sample Size

The sample size of this study is not determined based on statistical calculations, it is rather determined based on the probability of observing an adverse event (AE). A sample size of 8 subjects per cohort randomized in a 3:1 ratio to the study drug versus placebo represents a typical panel for a FIH study. This number of subjects is judged adequate to achieve the study objectives.

3.3 Treatment Assignment

All subjects enrolled will receive a single infusion of NS101, or matching placebo, under fasting conditions over a period of approximately 1 hour, at the target dose levels listed in the table below. Each subject's body weight on Day -1 will be used to calculate the dose required, based on their assigned dose level [body weight (kg) x dose level (mg) = administered dose (mg)].

Cohort Number	Dose level	Number of subjects receiving NS101	Number of subjects receiving placebo
1	0.25 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
2	0.75 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
3	1.50 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
4	3.0 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
5	6.0 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
6	12.0 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
7	24.0 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)
8	48.0 mg/kg	6 (1 sentinel, 5 non-sentinel)	2 (1 sentinel, 1 non-sentinel)

Note: Adjustments to the currently outlined dose levels may be made based on data from completed dose levels. However, the dose to be administered in a given cohort will not exceed the one currently outlined in the protocol.

3.4 Randomization

Randomization schedules will be generated through SAS® for Windows, Release 9.4 (SAS Institute Inc., Cary, NC, USA) software, prior to study execution. Block randomization will be used, and one randomization scheme will be produced for each cohort separately.

Subjects eligible for participation will be randomized (on Day 1) to receive NS101 or placebo in a 3:1 ratio, for a total of 6 subjects receiving NS101 ("active") and 2 subjects receiving matching placebo in each dose level. Each cohort will include 2 sentinel subjects randomized in a 1:1 ratio (1 active and 1 placebo) dosed initially, and the remaining 6 subjects (5 active and 1 placebo) will be dosed at least 48 hours later (each dosed at least 15 minutes apart).

Subjects assigned to Cohorts 5 through 8 will be randomized a second time for collection of a CSF sample at one of the following timepoints: 24, 36, 168, or 336 hours post-start of infusion. Each of the 4 timepoints will be assigned to 2 subjects. Sentinel subjects will be randomized to the same timepoint, to ensure that each timepoint is assigned to at least 1 subject receiving the active drug, NS101.

Due to the limited availability of the anesthesiologist performing the procedure, the assignment of timepoints for CSF collection, initially only determined by the randomization scheme, may be modified in consideration of each subject's dosing date and time. For example, 2 subjects allocated to one CSF timepoint may swap their timepoint with that of 2 subjects assigned to another timepoint. Importantly, although the attribution of timepoints will differ from the initial randomization scheme, the overall requirements outlined in the protocol in terms of number of subjects per timepoint (2 subjects), and assignment of sentinel subjects to the same timepoint, will be respected.

3.5 Blinding:

The subjects and the clinical personnel involved in the collection, monitoring, revision, or evaluation of AEs, or personnel who could have an impact on the outcome of the study will be blinded with respect to the subject's treatment assignment (NS101 or placebo). Blinding will be maintained until the database is locked and the analysis population are determined.

The randomization schedule for cohort 1 to 8 was provided according to randomization specification. Designated pharmacy personnel at the clinical site not directly involved with the clinical aspects of the trial will prepare and dispense the study medication and will be aware of the randomization code. The study drug and placebo will have the same visual appearance in order to avoid compromising the study blinding.

In the event of an emergency for an individual subject in which knowledge of the study treatment is critical to the subject's medical management or for the decision of dose escalation, the Investigator may break the blind for that subject. An envelope for each subject containing the treatment assignment will be available from the pharmacy personnel. The Investigator or other attending study physician will make every effort to contact the Sponsor prior to unblinding a subject's treatment assignment and will record the date and reason for unblinding in the study source documents.

As all samples (including placebos) will be analyzed, the randomization code will not be available to the Bioanalytical Division of Syneos Health prior to analysis from each cohort. However, since concentrations of NS101 above the analytical method's lower limit of quantification (LLOQ) should not be observed for subjects receiving the placebo, blinding of treatment assignments by personnel in the Bioanalytical Division involved with sample analysis cannot be assured completely.

Blinded PK results will be available during the course of the study. These results will be reported without revealing the subject's identity.

3.6 Subject Withdrawal and Replacement

Subjects who withdraw, or are withdrawn, from the study for safety and tolerability reasons after

dosing will not be replaced. However, subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability, may be replaced. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 10 subjects per cohort.

4. Endpoints

- Primary endpoints:
    - Incidence, nature, relatedness, and severity of AEs.
    - Changes in vital signs measurements, physical examination, clinical laboratory test findings, and 12-lead electrocardiogram (ECG) results.
- Secondary endpoints:
    - Serum PK parameters for NS101 will include the following:
        - area under the concentration-time curve, from time zero to 24-hours (AUC_{0-24})
        - area under the concentration-time curve from time zero until the last observed concentration (AUC_{0-t})
        - area under the concentration-time curve from time zero to infinity (AUC_{0-inf})
        - maximum observed concentration (C_{max})
        - time when the maximum concentration is observed (T_{max})
        - terminal elimination half-life ($T_{½\ el}$)
        - elimination rate constant (K_{el})
        - apparent body clearance (Cl)
        - apparent volume of distribution (V_z)
    - Concentrations of NS101 in CSF for Cohorts 5 to 8
    - Serum to CSF ratio of NS101 concentrations
    - Immunogenicity of NS101, measured as the number and percentage of subjects who develop detectable anti-drug antibody (ADA) and neutralizing antibody (NAb)
- Exploratory endpoints:
    - Effect (E): variation of level of FAM19A5 (Δ FAM19A5) following administration of NS101:
        - PD: Change in baseline of FAM19A5 levels
        - The following PD parameters in plasma:
            - $AUEC_{0-t}$
            - maximal observed effect (E_{max})
            - time of maximal observed effect (TE_{max})
        - Concentrations of FAM19A5 in CSF

5. Analysis Populations

All subjects' inclusion status into each analysis population will be determined after database lock.

5.1 Randomized Population

The randomized population is defined as all subjects who were randomized.

5.2 Safety Population

The safety population is defined as all subjects who received any amount of NS101 or placebo.

5.3 PK Concentration Population

The PK concentration population will include all subjects who received NS101 and have at least one post-dose concentration ≥ LLOQ.

5.4 PK Parameter Population

The PK parameter population will comprise all subjects who received any amount of NS101 and have sufficient post-dose serum concentration-time data to adequately determine at least one PK parameter. Any subject who has at least one major protocol deviation which might have an impact on the PK parameter analyses will be excluded from the PK population.

Here are some aspects to be considered (but not to be limited to) when determining data availability for the PK population: inclusion and exclusion criteria, acceptable times for visit dates and measurements, compliance with treatment, the nature and quality of the data, withdrawal, and any protocol deviation. The final responsibility of deciding which subjects are to be included or excluded lies with the PI and/or the sponsor.

5.5 CSF Population

The CSF population is defined as all subjects having received at least one dose of NS101 and CSF sample collected.

5.6 PD Population

The PD population will include all subjects having received at least one dose of NS101, as appropriate, and for whom at least one post-dose FAM19A5 parameter can be adequately characterized.

5.7 Immunogenicity Population

The immunogenicity population will include all subjects who have received any amount of NS101 and have at least one post-dose ADA/NAb measurement.

6. General Aspects for Statistical Analysis

6.1 General Methods

SAS for Windows, Release 9.4, software will be used to perform all data analyses.

All data in the database will be presented in the data listings. Unless otherwise stated, all listings will be sorted by randomized cohort, subject number, and assessment date and time. For the purposes of the summary tables, all subjects randomized to placebo will be pooled into a single placebo group.

The following labels for treatment will be used on all tabulations where the results are displayed by treatment, in the following order:

- All Placebo
- NS101 0.25 mg/kg
- NS101 0.75 mg/kg
- NS101 1.50 mg/kg
- NS101 3.0 mg/kg
- NS101 6.0 mg/kg
- NS101 12.0 mg/kg
- NS101 24.0 mg/kg
- NS101 48.0 mg/kg

6.2 Summary Statistics:

Unless otherwise stated, continuous variables will be summarized using the number of observations (n), and the statistics mean, median, standard deviation (SD), minimum (min) and maximum (max). The min and max values will be presented to the same number of decimal places as recorded in the case report form (CRF), median will be presented to one more decimal place than the raw data and the mean and SD will be presented to two more decimal places than the raw data. Summaries of change-from-baseline variables will include only subjects who have both a baseline value and corresponding value at the timepoint of interest. Categorical variables will be summarized with frequency counts and percentages. Percentages will be rounded to one decimal place, with the denominator being the number of subjects in the relevant population, unless otherwise stated.

For the serum and CSF PK and PD data, the data will be rounded to two decimal places in the listings, except for the following situations:

- Elimination rate constant (K_{el}) data: rounded off to four decimal digits
- PK parameters related to time, such as T_{max}, $K_{el\ Lower}$, and $K_{el\ Upper}$, will be reported with the same precision as the actual sampling time: rounded off to 3 decimal digits
- Concentration versus time data, as well as C_{max}: reported as they appear in the corresponding dataset

Summary statistics include the geometric mean (two decimal places) and coefficient of variation (CV)% (two decimal place). The geometric mean and CV% will not be calculated for T_{max}, $T_{½\ el}$, and K_{el}.

Only data from protocol scheduled ("nominal") visits / timepoints will be included in the summary tables. Data from unscheduled visits will not be included in the summary tables (unless they were used as baseline) but will be included in the listings.

In the case of a repeat test, both assessments will be presented in the listings. If a repeat measurement was performed due to the first measurement being outside the normal range for the given assessment and the second assessment confirms the first measurement, then only the first measurement will be used for analysis and the second assessment will be considered an unscheduled timepoint. In the same case, if the second assessment falls within normal ranges, then the second assessment will be used for analysis of that timepoint. In all other cases, the first measurement will be taken as the assessment to be used for the analysis.

6.3 Key Definitions

Baseline:

Unless stated otherwise, baseline will be defined for each subject and will be defined as the last non-missing measurement (including repeated and unscheduled assessments) before IV infusion of the study drug, NS101. Post-baseline will be considered as all measurements collected after end of IV infusion of the study drug, NS101. Unknown, Not Done, Not Applicable and other classifications of missing data will not be considered when calculating baseline observations unless the finding is a valid categorical observation.

Study day:

Study day will be calculated using study drug administration date as the reference date. If the date of interest occurs on or after the study drug administration date, study day will be calculated as follows: date of interest – study drug administration date + 1 = study day. If the date of interest occurs prior to the study drug administration date, study day will be calculated as follows: date of interest – study drug administration date = study day. There will be no study day 0.

Prior medication:

Prior medication is defined as any medication which has a start date before screening.

Concomitant medication:

Concomitant medication is defined as any medication which has a dosing date after screening and before the last study day.

Body mass index (BMI):

The BMI is a value derived from the mass (weight) and height of a person, calculated by the following equation: Weight (kg) / Height $(m)^2$ = BMI

Nominal timepoint value:

Nominal timepoint value is the value at a scheduled timepoint or the average of a set of values around a scheduled timepoint.

6.4 Drop-Outs and Missing Data

There will be no imputation for missing data, unless otherwise specified. Missing data shall be presented in subject listings as either “UN” for an unknown day, “UNK” for an unknown month, “UNKN” if year is unknown or “N/A” (not applicable), with the corresponding definition in the footnotes.

In the case of withdrawal of consent, all data from subjects who withdraw from the study will be included in all summaries up to the time of withdrawal. For all other withdrawals, all data captured will be included in the safety summaries.

For PK analysis, only observed concentration data will be used in the data analysis except for concentration values below the lower limit of quantification (BLQ). No attempt will be made to extrapolate or interpolate estimates for missing data.

6.5 Protocol Deviations

Subject data will be examined for evidence of protocol deviations. All protocol deviations will be categorized (Major, Minor, Critical or No impact) and listed by subject.

7. Subject Attributes

7.1 Subject Disposition

The number of subjects who were screened, enrolled, randomized, dosed, and completed or discontinued from the study, will be summarized. This summary table will also include the number of subjects in each analysis population. The data will be presented by cohort and overall (frequency and the percentage of subjects) and listed. If applicable, reason(s) for discontinuation will also be listed.

7.2 Inclusion and Exclusion Criteria

All recorded inclusion/exclusion criteria status will be presented in a data listing for the safety population. Each subject's inclusion or exclusion from each population will also be presented in a data listing.

7.3 Demographic and Baseline Characteristics

Demographic characteristics (including age, sex, ethnicity, race, country, and dominant hand) and baseline characteristics (body measurements of height, weight, and BMI) will be summarized by study treatment and listed by subject for the safety population. If the safety population and PK parameter population are different, then a separate table for the PK parameter population will be generated.

Descriptive statistics (n, mean, SD, min, median, and max) will be calculated for continuous variables using the last results obtained prior to study drug administration. Frequency counts and percentages will be tabulated for categorical variables.

7.4 Medical History

Medical history will be listed by subject. The latest version of the Medical Dictionary for Regulatory Activities (MedDRA, version 24.1) will be used to classify all medical history findings by system organ class (SOC) and preferred term (PT).

7.5 Medications

All prior and concomitant medications will be presented in data listings for the Randomized Population.

Concomitant medications will be coded using the latest version of the World Health Organization Drug Dictionary (WHOD). The total number of concomitant medications and the number and percentage of subjects with at least one concomitant medication will be summarized for the Safety Population. Concomitant medication data will be presented separately by anatomical therapeutic chemical (ATC) classification code (2^{nd} level), preferred term (PT), and treatment. Note: When 2^{nd} level classification code is not available, 1^{st} level classification will be used instead.

7.6 Study Drug Administration

The study drug administration details including cohort, date and time of administration, frequency, route, formulation, planned dose, amount of study drug administered, and fasting status, will be listed by subject.

8. Pharmacokinetic (PK) Analyses

All serum concentration and serum PK parameter summaries, and analyses will be conducted on the PK concentration population and the PK parameter population, respectively.

Serum concentrations, collection times, and collection time deviations will be listed for the PK concentration population by dose level. The serum concentrations of NS101 will be summarized for the PK concentration population by dose level, and time point, using descriptive statistics. The individual and mean (±SD) concentrations for NS101 will be presented graphically on both linear and semi logarithmic scales by dose level for the PK concentration population.

8.1 Data Presentation

For all PK analyses, concentration values BLQ that occur before the first measurable concentration of the study drug will be set to "0.00"; BLQ values that occur after first measurable concentration will be set to" missing". No imputations will be made on BLQ concentrations.

Invalid concentration values (due to bioanalytical or clinical issue) that occur prior to dosing will be replaced by "0.00". Invalid concentration values that occur after dosing will be set to "missing" for tabulation, graphical representation, and calculation purposes.

The actual clock time for dosing start and end and the actual clock time for each collection time for the PK samples will be recorded using electronic data capture. For all sampling times, the actual sampling duration will be calculated as the difference between the sample collection actual clock time and the actual clock time of dosing end. The actual post-dose sampling times, expressed in hours and rounded off to three decimal digits, will be used to calculate the PK parameters. Pre-dose samples will always be reported as "0.000", regardless of the time difference. Scheduled sampling times will be presented in concentration tables and mean graphs, while actual sampling times will be presented in the individual graphs in the PK section of the report. Actual sampling times also will be used for final PK parameter derivation. If actual sampling time is missing, then nominal time will be used. A listing of the actual times for PK sampling will be provided for PK samples.

8.2 Serum PK Sampling Schedule

A total of 21 blood samples will be collected in each cohort for serum PK analysis: pre-start of infusion and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 4, 6, 8, 12, 24, 36, 48, 96, 168, 336, 504, 672, and 1416 hours post-start of infusion.

8.3 Serum PK Parameters

Serum PK parameters will be presented in data listings and summarized in tables by dose level, using descriptive statistics (n, arithmetic mean, SD, CV%, geometric mean, min, median, and max).

The serum concentrations of NS101 will to be used to calculate the following parameters by standard non-compartmental methods:

Parameter	Definition
$AUC_{0\text{-}inf}$	area under the concentration-time curve from time zero to infinity (extrapolated), calculated as $AUC_{0\text{-}t}+C_t/\lambda_z$, where: C_t = the last measurable concentration

Parameter	Definition
AUC_{0-t}	area under the concentration-time curve from time zero until the last observed concentration
AUC_{0-24}	area under the concentration-time curve from time zero to time 24 hours
Cl	apparent body clearance, calculated as dose / AUC_{0-inf}
C_{max}	maximum observed concentration
K_{el}	elimination rate constant
$T_{½\ el}$	terminal elimination half-life, calculated as ln 2/ K_{el}
T_{max}	time when the maximum concentration is observed
Residual area	percentage of the area extrapolated for calculation of area under the concentration-time curve from time zero to infinity, calculated as $[(AUC_{0-inf} – AUC_{0-t}) / AUC_{0-inf}] * 100$
V_z	apparent volume of distribution, calculated as dose / (K_{el} * AUC_{0-inf})

AUC_{0-t}, AUC_{0-24}, and AUC_{0-inf} will be calculated using the linear trapezoidal method (linear up log down).

K_{el} is the elimination rate constant. This parameter will be the negative of the estimated slope of the linear regression of the ln-transformed serum concentration versus time profile in the terminal elimination phase. The Best-fit method, in Phoenix WinNonlin, will be used to calculate the K_{el} from at least 3 concentration data points excluding the C_{max}. Rsq adjusted, the goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of K_{el} must be ≥ 0.8. If the K_{el} cannot be measured (e.g.: fewer than 3 non-zero concentrations in the terminal elimination phase or Rsq adjusted < 0.8), the PK parameters derived from K_{el} (λ_z, $T_{½\ el}$, AUC_{0-inf}, Residual area, Cl and V_z) will be presented in the Listing(s) but excluded from descriptive statistics. The timepoint where ln-linear K_{el} calculation begins ($K_{el\ Lower}$) and the actual sampling time of the last measurable concentration used to estimate the K_{el} ($K_{el\ Upper}$), as well as the Rsq adjusted for the ln-linear regression for the calculation of the elimination rate constant will be reported.

If the Residual area is > 20%, the individual result will be flagged in the Listings as well as values for $T_{½\ el}$, AUC_{0-inf}, Cl, and V_z.

8.4 CSF PK Sampling Schedule

Subjects included in Cohorts 5 through 8 will have one CSF sample collected, via lumbar puncture, at one of the following timepoints: 24, 36, 168, or 336 hours post-start of infusion.

NS101 concentrations in CSF will be presented in the listings, as well as the ratio of NS101 in serum to NS101 in CSF at the applicable time-matched timepoint, i.e. serum NS101/CSF NS101 at time xx hrs.

8.5 Assessment of Dose Proportionality

An exploratory assessment of dose proportionality will be undertaken for the PK parameters AUC_{0-t}, AUC_{0-inf}, and C_{max}. The power model will be used and will include the PK parameter as the response variable and dose (mg) as the explanatory variable. For this model, the variable dose will be treated as a continuous variable. All cohorts will be considered for the analysis as a minimum of three doses are required. The form of the model is as follows:

PK Parameter = $e^{\alpha} \times \text{dose}^{\beta} \times e^{\epsilon}$, where dose ≥ 0, and e^{ϵ} represents the associated error.

Thus, perfect dose proportionality is met when β=1 (ignoring error). This becomes a linear relationship following a natural-log transformation, to which a linear regression will be fit by ordinary least squares (OLS):

ln(PK Parameter) = α + β × ln(dose) + ϵ where dose > 0, and ϵ represents the associated error.

The estimate of β, together with a 90% confidence interval (CI), will be provided (for each PK parameter model), and this will be used to quantify dose proportionality. According to Hummel et al. (2009), the following criterion may be used for exploratory dose proportionality evaluations: If the (two-sided) 90% CI for β is wholly contained within the interval, [1+ln(0.5)/ln(ρ), 1+ln(2)/ln(ρ)], then dose proportionality is suggested across the investigated dose range. Here, ρ is defined as the ratio of the highest to lowest dose. This interval criterion will be reported along with the corresponding 90% CI estimate for β (presented to three decimal places).

The SAS code for the analysis model will follow the format given below (using the mixed procedure to fit the linear regression). The input variables (or datasets) are depicted in italicized red text and have been given generic names.

```
proc mixed data=dataset order=data;
  model ln_pk_parameter = ln_dose / solution;
  estimate 'Beta Estimate' ln_dose 1 / cl alpha=0.1;
  ods output estimates = estimates;
run;
```

The PK parameter values estimated from the power model will be plotted against dose. This plot will also include individual subject values with the observed mean ± SD (PK parameter versus dose level).

8.6 Non-parametric Assessment of Tmax

A non-parametric assessment to test for central location differences among dose levels, will be undertaken for T_{max}. All cohorts will be considered for the analysis as a minimum of two dose levels are required.

An overall Kruskal-Wallis Test for differences in PK values across dose levels will be conducted (e.g., H_0: on average, T_{max} values are equal across dose levels, and H_a: on average, PK parameter values differ among at least two distinct dose levels). If the P-value is significant at the 5% level, then PK parameter values are considered unequal across all dose levels. If the P-value is not significant, then PK similarity may be suggested across the investigated dose range.

The SAS code to perform the test will follow the format given below (using the Npar1way procedure). The input variables, datasets and labels are depicted in italicized red text and have been given generic names.

```
proc npar1way data=dataset wilcoxon;
  class treatment;
  var pk_parameter;
  ods output KruskalWallisTest = Ktest;
run;
```

9. Pharmacodynamic Analyses

All plasma PD summaries and analysis will be conducted on the PD population.

Plasma PD will be evaluated through the variation of the normalized and actuallevel of FAM19A5 following administration of NS101. Normalized and actual FAM19A5 level, absolute changes and percent changes from normalized and actual baseline in FAM19A5 level will be summarized for the PD population by dose level, and timepoint, using descriptive statistics.

Absolute changes from normalized and actual baseline FAM19A5 levels (ΔFAM19A5) will be determined. Baseline is defined as the Day 1 pre-dose plasma PD sample. At each post-dose measurement of FAM19A5, the Day 1 pre-dose plasma PD sample will be subtracted to calculate Δ FAM19A5. For percent change from normalized and actual baseline ΔFAM19A5 will be divided by Day 1 pre-dose plasma PD sample and multiplied by 100.

9.1 Data Presentation

The actual clock time for dosing start and end and the actual clock time for each collection time for the PD samples will be recorded using electronic data capture. For all sampling times, the actual sampling times will be calculated as the difference between the sample collection actual clock time and the actual clock time of dosing end. The actual post-dose sampling times, expressed in hours and rounded off to three decimal digits, will be used to calculate the PD parameters. Pre-dose samples will always be reported as "0.000", regardless of the time difference.

9.2 Plasma PD Sampling Schedule

A total of 14 blood samples will be collected in each cohort for PD analysis: pre-dose (baseline) and 1, 4, 6, 12, 24, 36, 48, 96, 168, 336, 504, 672, and 1416 hours post-start of infusion.

9.3 Plasma PD Parameters

Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales for normalized and actual FAM19A5.

Normalized and actual plasma FAM19A5 and changes from baseline ΔFAM19A5, along with PD parameters will be presented in data listings and summarized in tables by treatment, using descriptive statistics (n, arithmetic mean, geometric mean, SD, CV%, min, max, and median).

The following PD parameters will be calculated for plasma normalized and actual FAM19A5:

Parameter	Definition
$AUEC_{0-t}$	area under the PD effect versus time curve from time zero until the last measurable timepoint
E_{max}	maximum observed effect
TE_{max}	time of E_{max}

$AUEC_{0-t}$, will be calculated using the linear trapezoidal method (linear up log down).

9.4 CSF PD Sampling Schedule

Subjects included in Cohorts 5 through 8 will have one CSF sample collected, via lumbar puncture, at one of the following timepoints: 24, 36, 168, or 336 hours post-start of infusion.

All FAM19A5 concentrations in CSF will be listed, as well as the ratio of FAM19A5 in serum to FAM19A5 in CSF at the applicable time-matched timepoint, i.e. serum FAM19A5 /CSF

FAM19A5 at time xx hrs.

10. Immunogenicity Analyses

All immunogenicity summaries, listings, and analysis will be conducted on the immunogenicity population. Listings will be provided for the immunogenicity population.

The number and percentage of subjects who develop detectable ADA and NAb will be summarized. The main PK parameters will be summarized by dose level and category of ADA status (at least one positive/negative). A listing showing immunogenicity test results from each three-tiered approach will be presented by treatment and visit.

The ADA assay will follow a three-tiered approach consisting of (i) screening assay, (ii) confirmatory assay, and (iii) titration. Samples that are "confirmatory" in the screening assay will undergo further testing in the confirmatory assay to determine if subjects are true positive. The overall ADA assay result will be "positive" or "negative". For further characterization, the antibody level will be assessed by titration in confirmed positive samples. The confirmed positive samples in the ADA assay will be analyzed further to conduct a NAb assessment. The test outcome for NAb assay will be "positive" or "negative".

The results of the final ADA assay and the NAb assay will be summarized. The number and percentage of subjects will be provided by dose level and visit. The descriptive statistics of ADA titration results for each treatment group will be presented by visit. The number and percentage of subjects with at least one positive ADA assay and the number and percentage of subjects with at least one positive NAb assay status after study drug administration (including unscheduled visit and Study Exit visit) will also be presented in a table. Vertical bar plots with percentage of subjects with positive ADA results will be provided.

If positive ADA positive results are available, an overall summary table of TEAE will be provided for ADA positive vs negative results.

Boxplots will present PK levels for each dose level by ADA status.

10.1 Immunogenicity Sampling Schedule

A total of 6 immunogenicity blood samples will be collected: pre-start of infusion and at 96, 336, 504, 672, and 1416 hours post-start of infusion.

11. Safety

Safety and tolerability analysis will be performed for all subjects in the Safety Population. Safety and tolerability data will be reported using descriptive statistics. No inferential statistical analysis of safety data is planned.

Safety and tolerability to NS101 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), vital signs, 12-lead ECG, clinical laboratory parameters, weight, and physical examination. treatment-emergent adverse events (TEAEs) will be tabulated by study treatment. Changes from baseline values in vital signs, ECG, and clinical laboratory parameters tabulated by study treatment will be evaluated.

11.1 Extent of Exposure

Study drug administration will be listed. The exposure to each dose will be summarized by treatment and overall.

11.2 Adverse Events (AEs)

AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be grouped by system organ class (SOC) and PT and summarized by actual treatment. The summary tables will present the number and percentage of total subjects and number of events by SOC and by PT.

An AE is defined as any untoward medical occurrence in a clinical study subject after providing written informed consent for participation in the study that does not necessarily have a causal relationship with the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product.

The severity of AEs will be graded according to criteria from the latest version of the Common Terminology Criteria for Adverse Events (CTCAE). The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE. Each AE must be classified based on medical judgment and according to the following relationship categories: probable, possible, remote/unlikely, and unrelated.

All AE summaries will include TEAEs only. TEAEs are defined as AEs that commence on or after the time of first study drug administration. AEs without an onset date or time or AEs with an onset date of the date of first study drug administration but without an onset time will be defined as treatment emergent, except if an incomplete date (e.g., month and year) clearly indicates that the event started prior to administration of first study drug or if the AE stop date indicates that the event started and stopped prior to administration of first study drug.

TEAEs will be summarized by treatment. The number and percentage of subjects experiencing AEs and the number of TEAEs will be tabulated. Subjects who experience the same AE (in terms of MedDRA PT) more than once will only be counted once for that event, however, the total number of events will also be counted per category. This also applies to sub-categories displayed in the summaries.

The following summaries will be presented:

- Overall summary of TEAEs
- TEAEs by SOC and PT
- TEAEs by SOC, PT, and severity
- TEAEs by SOC, PT, and relationship to study drug
- Serious TEAEs by SOC and PT
- TEAEs with overall occurrence greater than 5% by SOC and PT

All AEs will be listed. This will include a separate listing of serious AEs (SAEs) and Non-TEAEs.

11.3 Clinical Laboratory Evaluations

The following clinical laboratory evaluations will be performed: biochemistry, hematology, coagulation, serology, and urinalysis.

Clinical laboratory values will be flagged as either high or low based on the reference ranges provided by the laboratories for each laboratory parameter.

Actual values and changes from baseline for quantitative clinical laboratory test results will be summarized by cohort/treatment for each time point using descriptive statistics (n, mean, SD, min, median, and max). Unscheduled visits will not be included in the summary tables (unless they were used as baseline).

Shift tables from baseline to each scheduled post-baseline visit, will be generated for categorical clinical laboratory test results using normal; abnormal (NCS, CS), or missing categories as appropriate by cohort/treatment for each time point for the safety set.

Individual clinical laboratory results and reference ranges will be presented in data listings. Unscheduled visits will be included in data listings.

11.4 Vital Signs

Actual values and change from baseline will be summarized by cohort/treatment at each time point using descriptive statistics. Classifications of findings will be shown in a shift table with changes from baseline to post baseline, in addition to being included in the listings. The classifications of findings include “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant”. Orthostatic vital signs were collected for Cohort 1 to 4. Orthostatic vital signs collection stopped due to Protocol Amendment II and only screening visit data for subject 4001 to 4005 are applicable for cohort 4. These data will be listed separately.

11.5 12-Lead ECG

Actual values and change from baseline for the ECG parameters will be summarized by cohort/treatment at each timepoint using descriptive statistics.

Findings of 12-lead ECGs will be classified as either “normal”, “abnormal, not clinically significant”, or “abnormal, clinically significant”. A shift table for categorical variables will also be created.

All safety ECG data will be listed.

11.6 Physical Examination

A complete physical examination includes assessments of the following: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination.

A brief physical examination includes assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject.

All physical examination data will be listed. Any abnormal finding noted after dosing will be documented as an AE if judged as a clinically significant change from baseline.

11.7 Drug and Alcohol Screens

Results for drug and alcohol screens will be presented in a separate listing for the Safety Population.

11.8 Sheehan Suicidality Tracking Scale (S-STS)

A listing of S-STS Total score will be provided.

11.9 Injection Site Evaluation

Injection site evaluation scores will be tabulated with frequency counts and percentages. A listing of injection site evaluation data will be provided. Bleeding or fluid loss at the injection site will be documented.

11.10 Pulse Oximetry

Pulse Oximetry data will be listed.

11.11 COVID-19 Visit Impact

COVID-19 Visit Impact data will be listed.

12. Changes from Analysis Planned in Protocol

None.

13. Reference List

- Hummel J., McKendrick S., Brindley C. and French, R. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. Pharmaceut. Statist. 2009; 8: 38-49.

14. Programming Considerations

- All TLFs, and statistical analyses will be generated using SAS for Windows, Release 9.4.
- Phoenix® WinNonlin® version 8.3.4 (Certara USA, Inc., Princeton, NJ) will be used for all PK analyses. This software was validated by Syneos in compliance with US 21 CFR Part 11 regulation.

14.1 General Considerations

- One SAS program can create several outputs.
- Each output will be stored in a separate file.
- Output files will be delivered in rich text format (RTF) that can be manipulated in MS Word.
- Numbering of TLFs will follow International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline E3 on Structure and Content of Clinical Study Reports, which was approved in 1996. Table, Listing, and Figure Format.

14.1.1 General

- All TLFs will be produced in landscape format, unless otherwise specified.
- All TLFs will be produced using the Times New Roman font, size 10. The font size may be reduced as necessary to allow additional columns to be presented, but not at the expense of clarity. The orientation may be changed to portrait, if appropriate.
- The data displays for all TLFs will have a minimum blank 1-inch margin on all 4 sides.
- Headers and footers for figures will be in Times New Roman font, size 10.
- Legends will be used for all figures with more than 1 variable, group, or item displayed.
- TLFs will be in black and white (no color), unless otherwise specified
- Specialized text styles, such as bolding, italics, borders, shading, and superscripted and subscripted text, will not be used in the TLFs, unless otherwise specified. On some occasions, superscripts 1, 2, or 3 may be used (see below).
- Only standard keyboard characters will be used in the TLFs. Special characters, such as non-printable control characters, printer-specific, or font-specific characters, will not be used. Hexadecimal-derived characters will be used, where possible, if they are appropriate to help display math symbols (e.g., μ). Certain subscripts and superscripts (e.g., cm_2, C_{max}) will be employed on a case-by-case basis.
- Mixed case will be used for all titles, footnotes, column headers, and programmer-supplied formats, as appropriate.

14.1.2 Headers

- All output should have the following header at the top left of each page: “Protocol NS101_P1_01 (200242)”.
- All output should have Page n of N at the top or bottom right corner of each page. TLFs are internally paginated in relation to the total length (i.e., the page number should appear sequentially as page n of N, where N is the total number of pages in the table).
- The SAS system date and time output was generated should appear along with the program name as a footer on each page.

14.1.3 Display Titles

Each TFL is identified by the designation and a numeral (e.g., Table 14.1.1). ICH E3 numbering is strongly recommended, but Sponsor preferences are obtained before final determination. A decimal system (x.y and x.y.z) are used to identify TLFs with related contents. The title is left-justified. The analysis set are identified on the line immediately following the title. The title and table designation are single spaced. A solid line spanning the margins will separate the display titles from the column headers. There will be 1 blank line between the last title and the solid line.

For example:

Table x.y.z
First Line of Title
Second Line of Title

14.1.4 Column Headers

- Column headings are displayed immediately below the solid line described above in initial upper-case characters.
- In the case of efficacy tables, the variable (or characteristic) column will be on the far left followed by the treatment group columns and total column (if applicable). P-values may be presented under the total column or in separate p-value column (if applicable). Within-treatment comparisons may have p-values presented in a row beneath the summary statistics for that treatment.
- For numeric variables, include “unit” in column or row heading when appropriate.
- Analysis set sizes will be presented for each treatment group in the column heading as (N=xx) (or in the row headings, if applicable). This is distinct from the ‘n’ used for the descriptive statistics representing the number of subjects in the analysis set.
- The order of treatments in the tables and listings will be Placebo first in the case of placebo-controlled studies and Active comparators first in the case of active comparator trials, followed by a total column (if applicable).

14.1.5 Body of the Data Display

14.1.5.1 General Conventions

Data in columns of a table or listing are formatted as follows:

- Alphanumeric values are left-justified
- Whole numbers (e.g., counts) are right-justified

14.1.5.2 Table Conventions

- Units will be included where available
- If the categories of a parameter are ordered, then all categories between the maximum and minimum category are presented in the table, even if n=0 for all treatment groups in a given category that is between the minimum and maximum level for that parameter. For example, the frequency distribution for symptom severity would appear as:

Severity Rating	N
Severe	0
Moderate	8
Mild	3

Where percentages are presented in these tables, zero percentages will not be presented and so counts of 0 will be presented as 0 and not as 0 (0%).

- If the categories are not ordered (e.g., Medical History, Reasons for Discontinuation from the Study, etc.), then only those categories for which there is at least 1 subject represented in 1 or more groups are included.
- An Unknown or Missing category are added to each parameter for which information is not available for 1 or more subjects.
- Unless otherwise specified, the estimated median for a set of values are printed out to 1 more significant digit than the original values, and mean and standard deviations are printed out to 2 more significant digits than the original values. The minimum and maximum should report the same significant digits as the original values. For example, for systolic blood pressure:

N	XX
Mean	XXX.XX
Std Dev	X.XX
Median	XXX.X
Minimum	XXX
Maximum	XXX

- P-values are output in the format: “0.xxx”, where xxx is the value rounded to 3 decimal places. Every p-value less than 0.001 will be presented as <0.001. If the p-value are less than 0.0001, then present as <0.0001. If the p-value is returned as >0.999, then present as >0.999

- Percentage values are printed to one decimal place, in parentheses with no spaces, one space after the count (e.g., 7 (12.8%), 13 (5.4%)). Predetermine how to display values that round down to 0.0. A common convention is to display as '<0.1', or as appropriate with additional decimal places. Unless otherwise noted, for all percentages, the number of subjects in the analysis set for the treatment group who have an observation will be the denominator. Percentages after zero counts should not be displayed and percentages equating to 100% are presented as 100%, without decimal places.
- Tabular display of data for medical history, prior/concomitant medications, and all tabular displays of adverse event data are presented by the body system, treatment class, or SOC with the highest occurrence in the active treatment group in decreasing order, assuming all terms are coded. Within the body system, drug class and SOC, medical history (by PT), drugs (by ATC code), and adverse events (by preferred term) are displayed in decreasing order. If incidence for more than 1 term is identical, they should then be sorted alphabetically. Missing descriptive statistics or p-values which cannot be estimated are reported as "-".
- The percentage of subjects is normally calculated as a proportion of the number of subjects assessed in the relevant treatment group (or overall) for the analysis set presented. However, careful consideration is required in many instances due to the complicated nature of selecting the denominator, usually the appropriate number of subjects exposed. Describe details of this in footnotes or programming notes.
- For categorical summaries (number and percentage of subjects) where a subject can be included in more than one category, describe in a footnote or programming note if the subject is included in the summary statistics for all relevant categories or just 1 category and the criteria for selecting the criteria.
- Where a category with a subheading (such as system organ class) must be split over more than one page, output the subheading followed by "(cont)" at the top of each subsequent page. The overall summary statistics for the subheading should only be output on the first relevant page.

14.1.5.3 Listing Conventions

- Listings will be sorted for presentation in order of treatment groups as above, subject number, visit/collection day, and visit/collection time.
- Missing data are represented on subject listings as either a hyphen ("-") with a corresponding footnote ("- = unknown or not evaluated"), or as "N/A", with the footnote "N/A = not applicable", whichever is appropriate.
- Dates are printed in SAS DATE9.format ("ddMMMyyyy": 01JUL2000). Missing portions of dates are represented on subject listings as dashes (--JUL2000). Dates that are missing because they are not applicable for the subject are output as "N/A", unless otherwise specified.

- All observed time values are to be presented using a 24-hour clock HH:MM or HH:MM:SS format (e.g., 11:26:45, or 11:26). Time will only be reported if it was measured as part of the study.
- Units will be included where available

14.1.5.4 Figure Conventions

- Unless otherwise specified, for all figures, study visits will be displayed on the X-axis and endpoint (e.g., treatment mean change from baseline) values will be displayed on the Y-axis.

14.1.6 Footnotes

- A solid line spanning the margins will separate the body of the data display from the footnotes.
- All footnotes will be left-justified with single spacing immediately below the solid line underneath the data display.
- Footnotes will always begin with “Note:”, if an informational footnote, or 1, 2, 3, etc., if a reference footnote. Each new footnote will start on a new line, where possible.
- Subject specific footnotes are avoided, where possible.
- Footnotes will be used sparingly and add value to the table, figure, or data listing. If more than six lines of footnotes are planned, then a cover page may be used to display footnotes, and only those essential to comprehension of the data will be repeated on each page.
- The last line of the footnote section will be a standard source line that indicates the name of the program used to produce the data display and date the program was run (i.e., ‘Program: myprogram.sas’).

15. Quality Control

SAS programs are developed to produce output such as analysis data sets, summary tables, data listings, figures or statistical analyses. These will be developed and undergo quality control in accordance with the latest versions of SOP 2800 and SOP 2801.

End of document