Statistical Analysis Plan

A Phase 1, First Time in Human Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of NST-6179 in Healthy Subjects

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LIST OF ABBREVIATIONS

Abbreviations pertain to the statistical analysis plan (SAP) only (not the tables, figures, and listings [TFLs]).

$\%AUC_{extrap}$	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
ADaM	Analysis Data Model
AE	adverse event
Ae_{t1-t2}	amount of the dose administered recovered over the time interval t1 to t2
ANOVA	analysis of variance ANOVA
AUC_{0-24}	area under the concentration-time curve from time 0 to 24 hours postdose
AUC_{0-72}	area under the concentration-time curve from time 0 to 72 hours postdose
$AUC_{0-\infty}$	area under the concentration-time curve from time 0 extrapolated to infinity
$AUC_{0-tlast}$	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
$AUC_{0-\tau}$	area under the concentration-time curve over a dosing interval
BLQ	below the limit of quantification
CDISC	Clinical Data Interchange Standards Consortium
CI	confidence interval
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum observed concentration
C_{min}	minimum observed concentration in a dosing interval
COVID-19	coronavirus disease 2019
CSR	clinical study report
CV	coefficient of variation
$DAUC_{0-\infty}$	$AUC_{0-\infty}$ normalised by dose administered
$DAUC_{0-tlast}$	$AUC_{0-tlast}$ normalised by dose administered
$DAUC_{0-\tau}$	$AUC_{0-\tau}$ normalised by dose administered
DC_{max}	C_{max} normalised by dose administered
DMP	data management plan
ECG	electrocardiogram
eCRF	electronic case report form
fe_{t1-t2}	percentage of the dose administered recovered over the time interval t1 to t2
GLSM	geometric least squares mean

ICH	International Council for/Conference on Harmonisation
ln	natural log
MedDRA	Medical Dictionary for Regulatory Activities
NA	not applicable
PK	pharmacokinetic(s)
QD	once daily
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
R^2-adj	adjusted coefficient for determination of exponential fit
$RA_{AUC0-\tau}$	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	observed accumulation ratio based on C_{max}
SAP	statistical analysis plan
SD	standard deviation
SDV	source document verification
$t_{1/2}$	apparent terminal elimination half-life
TEAE	treatment-emergent adverse event
TFL	table, figure, and listing
t_{last}	time of the last quantifiable concentration
t_{max}	time of the maximum observed concentration
V_z/F	apparent volume of distribution during the terminal phase
WHODrug	World Health Organization Drug Dictionary
λ_z	apparent terminal elimination rate constant
λ_z Lower	start of exponential fit
λ_z N	number of data points included in the log-linear regression
λ_z Span Ratio	time period over which λ_z was determined as a ratio of $t_{1/2}$
λ_z Upper	end of exponential fit

1. INTRODUCTION

This SAP has been developed after review of the clinical study protocol (Final Version 4 dated 06 October 2021) and electronic case report form (eCRF). Noting that later clinical study protocol (Final Version 5 dated 11 July 2022) was generated but never used for the study conduct.

This SAP describes the planned analysis of the pharmacokinetic (PK) safety, and tolerability data from this study. A detailed description of the planned TFLs to be presented in the clinical study report (CSR) is provided in the accompanying TFL shells document.

Continuous 12-lead electrocardiogram (ECG) data will be collected and stored for the future assessment of the NST-6179 concentration QT interval corrected for heart rate (QTc) response relationship following single oral doses of NST-6179. This data is not planned to be analysed by Labcorp Drug Development. If it decided to perform analysis on this data, this will be reported separately.

In general, the analyses are based on information from the protocol, unless they have been modified by agreement with NorthSea Therapeutics B.V. A limited amount of information about this study (eg, objectives, study design) is given to help the reader's interpretation.

This SAP must be finalised prior to any unblinding of study data for analysis purposes (interim or final). Additionally, the SAP and TFL shells should be finalised prior to any programming activities commencing.

This SAP supersedes any statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. If additional analyses are required to supplement the planned analyses described in this SAP, they may be performed and will be identified accordingly in the CSR. Any substantial deviations from this SAP will be agreed with NorthSea Therapeutics B.V and identified in the CSR.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonisation (ICH) E3 guideline *Structure and Content of Clinical Study Reports*, ICH E8 guideline *General Considerations for Clinical Trials*, and ICH E9 guideline *Statistical Principles for Clinical Trials*.[1,2,3]

The document history is presented in Appendix 1.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is:

- to assess the safety and tolerability of single and multiple oral doses of NST-6179 in healthy male and female subjects

2.2. Secondary Objective

The secondary objective of the study is:

- to evaluate the single and multiple oral dose PK of NST-6179 in healthy male and female subjects

2.3. Exploratory Objectives

The exploratory objectives of the study are:

- to collect data to assess the relationship between NST-6179 concentrations and QT interval corrected for heart rate (QTc) in healthy male and female subjects
- to evaluate the metabolite profile of NST-6179 in healthy male and female subjects.

3. STUDY ENDPOINTS

3.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

- incidence and severity of adverse events (AEs)
- incidence of laboratory abnormalities, based on haematology, clinical chemistry, coagulation, and urinalysis test results
- 12-lead ECG parameters
- vital signs measurements
- physical examinations.

3.2. Secondary Endpoints

The PK outcome endpoints of NST-6179 for Part A (single ascending dose in healthy subjects) are as follows:

- area under the concentration-time curve (AUC) from time 0 extrapolated to infinity ($AUC_{0-\infty}$)
- $AUC_{0-\infty}$ normalised by dose administered ($DAUC_{0-\infty}$)
- AUC from time 0 to the time of the last quantifiable concentration ($AUC_{0-tlast}$)
- $AUC_{0-tlast}$ normalised by dose administered ($DAUC_{0-tlast}$)
- maximum observed concentration (C_{max})
- C_{max} normalised by dose administered (DC_{max})

- time of the maximum observed concentration (t_{max})
- apparent terminal elimination half-life ($t_{1/2}$)
- apparent total clearance (CL/F)
- apparent volume of distribution during the terminal phase (V_z/F)
- amount of the dose administered recovered over the time interval t1 to t2 (Ae_{t1-t2})
- percentage of the dose administered recovered over the time interval t1 to t2 (fe_{t1-t2})
- renal clearance (CL_R).

The PK outcome endpoints of NST-6179 for Part B (multiple ascending dose in healthy subjects) are as follows:

- AUC over a dosing interval ($AUC_{0-\tau}$)
- $AUC_{0-\tau}$ normalised by dose administered ($DAUC_{0-\tau}$)
- $AUC_{0-\infty}$ (Day 1 only)
- $DAUC_{0-\infty}$ (Day 1 only)
- C_{max}
- DC_{max}
- t_{max}
- $t_{1/2}$
- CL/F
- V_z/F
- minimum observed concentration in a dosing interval (C_{min})
- observed accumulation ratio based on $AUC_{0-\tau}$ ($RA_{AUC0-\tau}$)
- observed accumulation ratio based on C_{max} (RA_{Cmax}).

Other PK parameters may also be added.

3.3. Exploratory Endpoints

Continuous 12-lead ECG data will be stored for the future assessment of the NST-6179 concentration-QTc response relationship following single oral doses of NST-6179.

For the highest planned dose group in Part B, additional samples will be taken for the identification of metabolites of NST-6179.

4. STUDY DESIGN

This will be a partly double-blind, randomised, placebo-controlled, single and multiple oral dose study conducted in 2 parts. Part A and Part B will be double-blind, randomised, placebo-controlled, with subjects receiving single (Part A) and multiple (Part B) oral doses.

4.1. Part A

Part A will comprise a double-blind, single-ascending dose, sequential-group design. Overall, 48 subjects will be studied in 6 groups (Groups A1 to A6), with each group consisting of 8 subjects.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Each subject will participate in 1 treatment period only. Subjects will reside at the study site from Day -1 (the day before dosing) to Day 4 of each treatment period, as applicable.

All subjects will return for a follow-up visit 10 to 14 days after their final dose.

Based on the ongoing review of the safety, tolerability, and PK results, additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 per period and will not extend beyond 28 days after each final dosing occasion.

In each of Groups A1 to A6, 6 subjects will receive NST-6179 and 2 subjects will receive placebo.

Groups A1 to A6
It is planned for each subject in Groups A1 to A6 to receive only a single dose of NST-6179 or placebo during the study. Doses will be administered in the fasted state in accordance with a randomisation schedule on the morning of Day 1.

Additional Groups (Groups A7 to A9)
If it is decided to enrol additional groups, fasting requirements, meal compositions, and timing of doses will be determined following review of the available PK data.

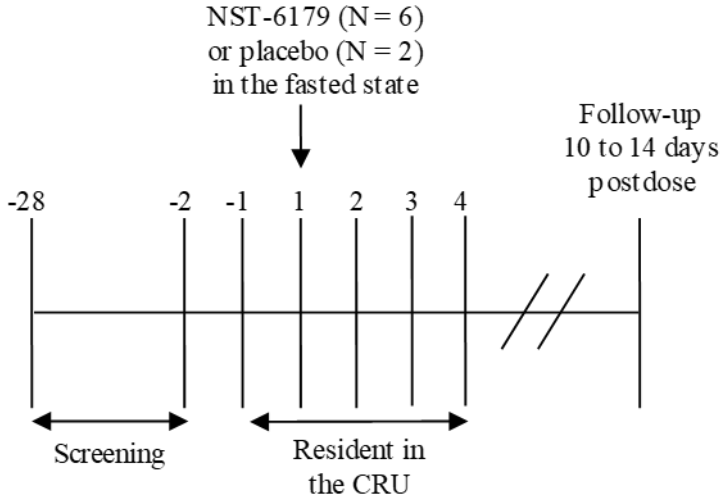
Sentinel Dosing
All groups in Part A will be divided into 2 sub-groups, with each sub-group being dosed 24 hours apart. The first sub-group will comprise 2 subjects, with 1 subject receiving NST-6179 and 1 subject receiving placebo. The second sub-group will comprise 6 subjects, with 5 subjects receiving NST-6179 and 1 subject receiving placebo.

There will be a minimum of 7 days between dose escalations for each group in Part A.

An overview of the study design is shown in Figure 1 and the planned dose levels are presented in Figure 2.

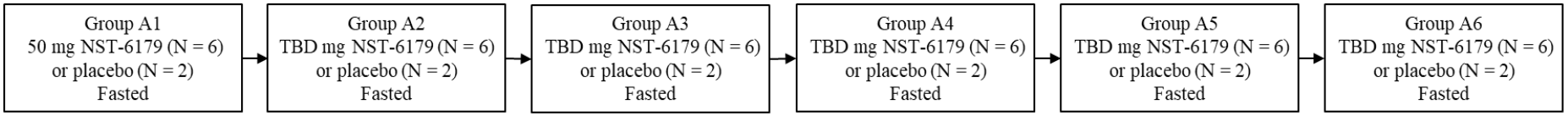
The total planned duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 6 weeks.

Figure 1: Study Design (Part A)



Abbreviations: CRU = Clinical Research Unit; N = number of subjects.

Figure 2: Planned Dose Levels (Part A)



Abbreviations: N = number of subjects; PK = pharmacokinetic(s); TBD = to be determined.
NOTE: dose levels may be adjusted based on the ongoing review of the safety, tolerability, and PK data. Doses will be administered in an escalating manner following satisfactory review by the sponsor and investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose) from the previous dose group

4.2. Part B

Part B will comprise a double-blind, multiple-ascending dose, sequential-group study design. Overall, 40 subjects will be studied in 4 groups (Groups B1 to B4), with each group consisting of 10 subjects. Part B may start in parallel with Part A provided the exposure is not predicted to exceed an exposure shown to be safe and well tolerated in Part A.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Each subject will participate in 1 treatment period only and reside at the study site from Day -1 until the morning of Day 16.

All subjects will return for a follow-up visit 10 to 14 days after their final dose.

Based on the ongoing review of the safety, tolerability, and PK results, additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 per period and will not extend beyond 28 days after each final dosing occasion.

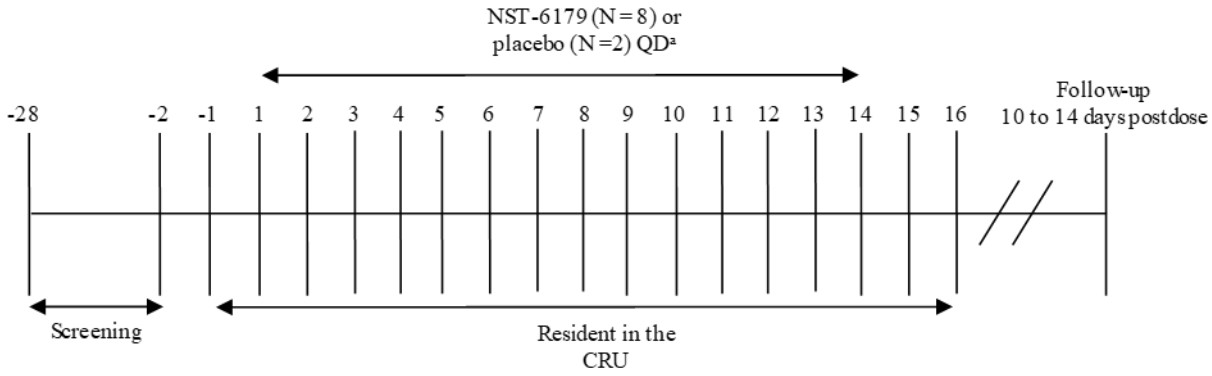
In each of Groups B1 to B4, 8 subjects will receive NST-6179 and 2 subjects will receive placebo. For all subjects, dosing is planned to be once daily (QD) on Days 1 to 14, inclusive. However, dosing frequency and duration in Part B may be changed following review of data from groups in Part A or earlier groups in Part B. The dose regimen will comprise no less than once every 2 days and will not exceed 4-times-daily dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days of dosing. There will be a minimum of 14 days between dose escalations for each group in Part B. The total daily dose administered will not be predicted to exceed an exposure shown to be safe and well tolerated in Part A.

Up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B.

An overview of the study design is shown in Figure 3, and the planned dose levels are presented in Figure 4.

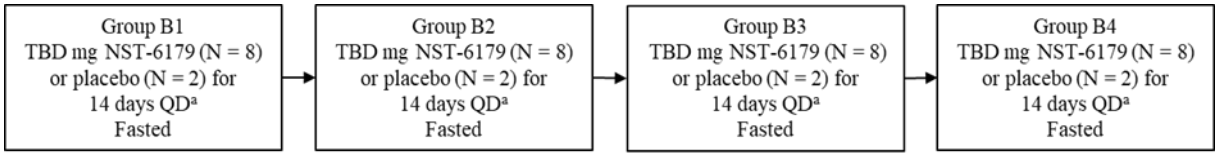
The total planned duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 8 weeks.

Figure 3: Study Schematic (Part B)



Abbreviations: CRU = Clinical Research Unit; N = number of subjects; PK = pharmacokinetic(s); QD = once daily.
NOTE: doses will be administered in an escalating manner following satisfactory review by the sponsor and investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose) from the previous dose group.
a. Dosing is planned to be QD on Days 1 to 14, inclusive. The dose regimen will comprise no less than once every 2 days and will not exceed 4-times-daily dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days of dosing.

Figure 4: Planned Dose Levels (Part B)



Abbreviations: N = number of subjects; PK = pharmacokinetic(s); QD = once daily.
NOTE: doses will be administered in an escalating manner following satisfactory review by the sponsor and investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose) from the previous dose group.
a. Dosing is planned to be QD on Days 1 to 14, inclusive. The dose regimen will comprise no less than once every 2 days and will not exceed 4-times-daily dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days of dosing.

5. SAMPLE SIZE JUSTIFICATION

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time NST-6179 is being administered to humans. However, the number of subjects in each part of the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study.

6. STUDY TREATMENTS

The study treatment names and ordering to be used in the TFLs for Part A are presented in Table 1 and for Part B in Table 2.

Table 1: Presentation of Study Treatments in TFLs (Part A)

Group	Study Treatment	Order in TFLs
A1 to A9	Placebo[a]	1
A1	TBD mg NST-6179	2
A2	TBD mg NST-6179	3
A3	TBD mg NST-6179	4
A4	TBD mg NST-6179	5
A5	TBD mg NST-6179	6
A6	TBD mg NST-6179	7
A7[b]	TBD mg NST-6179	8
A8[b]	TBD mg NST-6179	9
A9[b]	TBD mg NST-6179	10

[a] Placebo will be pooled across all groups
[b] Optional group

Table 2: Presentation of Study Treatments in TFLs (Part B)

Group	Study Treatment	Order in TFLs
B1 to B7	Placebo[a]	1
B1	TBD mg NST-6179 (QD)	2
B2	TBD mg NST-6179 (QD)	3
B3	TBD mg NST-6179 (QD)	4
B4	TBD mg NST-6179 (QD)	5
B5[b]	TBD mg NST-6179 (QD)	6
B6[b]	TBD mg NST-6179 (QD)	7
B7[b]	TBD mg NST-6179 (QD)	8

Abbreviations: QD = once daily
[a] Placebo will be pooled across all groups
[b] Optional group

All TFLs will be based on actual treatments (eg, if subject was assigned to receive placebo but was wrongfully dosed with active treatment they would be summarised and listed under active treatment).

All dose levels described above are the potential dose levels, and therefore are subject to change. The TFLs will reflect the dose levels utilised in the study, and these will be displayed in increasing order.

7. DEFINITIONS OF POPULATIONS

Any protocol deviations, including those due to coronavirus disease 2019 (COVID-19) and related restrictions (see Section 8.1.1), will be considered prior to database lock for their importance and taken into consideration when assigning subjects to populations.

7.1. All Subjects Population

The all subjects population will include all subjects who signed the informed consent form and had any study assessment recorded in the database per the protocol.

7.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (NST-6179 or placebo).

7.3. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of active study treatment (NST-6179) and have at least 1 valid PK concentration.

8. STATISTICAL METHODOLOGY

8.1. General

Listings will be provided for all data captured in the database, with the exception of medical history. Listings will include all subjects assigned to the all subjects population and include data up to the point of study completion or discontinuation. Subjects are generally considered to have completed the study if they complete the scheduled follow-up visit (rather than early termination visit). Any subject who discontinues the study will be identified accordingly in the listings. Summaries will include the subjects assigned to the relevant population based on data type.

Data analysis will be performed using the SAS® statistical software package Version 9.4 (or higher if a new version is issued during the study).

Analysis Data Model (ADaM) datasets will be prepared using Clinical Data Interchange Standards Consortium (CDISC) ADaM Version 2.1 (or higher if a new version is issued during the study) and CDISC ADaM Implementation Guide Version 1.1 (or higher if a new version is issued during the study). Pinnacle 21 Community Validator Version 4.0.1 (or higher if a new version is issued during the study) will be utilised to ensure compliance with CDISC standards.

For all statistical analyses, the hypothesis testing will be 2-sided and carried out on 0.05 significance level, unless specifically stated otherwise.

Caution should be used when interpreting results from the statistical analyses conducted in this study because the sample size is not based on power calculations.

Where reference is made to ‘valid’ data, this refers to non-missing data which meet the predetermined criteria (eg, are not flagged for exclusion).

Where reference is made to ‘all calculations’, this includes, but is not limited to, summary statistics, statistical analyses, baseline derivation, and changes from baseline.

All figures will be produced on linear-linear or discrete-linear scales, as applicable, unless specifically stated otherwise.

8.1.1. Handling of Data Quality Issues Due to Coronavirus Disease 2019 and Related Restrictions

Due to COVID-19 and related restrictions, there is a high risk for impact to data integrity, with the recognised potential for:

- Missed visits, caused by, for example:
  - Subject unable to travel to site due to restrictions, the need to quarantine, or COVID-19 infection
  - Subject unwilling to go to site due to fear of COVID-19 infection
  - Site postponing subject's visit due to investigator not being available (eg, if they have been dispatched to hospital handling COVID-19 infections)
- Site unable to replenish supply of investigational product
- Incomplete data entry by sites due to limited resources to support study or no access to source documents or to eCRF
- Outstanding source document verification (SDV) due to sponsor or country restrictions on remote SDV, or no or limited access to site(s) for on-site visits
- Unanswered queries

At the time of the reporting of the study results, all protocol deviations due to COVID-19 or related restriction will be assessed for their severity and impact on the analyses. If needed, appropriate statistical methods will be applied as a mitigating action (eg, data might be categorised into 2 analysis groups, with and without COVID-19 and related restrictions impact); however, this will exclude any imputations of the missing values. Any mitigating actions will be agreed with NorthSea Therapeutics B.V. in advance and identified in the CSR.

8.1.2. Calculation of the Summary Statistics

For continuous data the following rules will be applied:

- Missing values will not be imputed, unless specifically stated otherwise.
- Unrounded data will be used in the calculation of summary statistics.
- If the number of subjects with valid observations (n) <3, summary statistics will not be calculated, with the exception of n, minimum, and maximum.

- In general, as discontinuation data are not associated with any scheduled timepoint, they will be excluded from all calculations of summary statistics and statistical analyses. Exceptions may be made where justified.

For categorical data the following rules will be applied:

- For ordered categorical data (eg, AE severity), all categories between the possible minimum and maximum categories will be included, even if n = 0 for a given category.
- For non-ordered categorical data (eg, race), only those categories for which there is at least 1 subject represented will be included; unless specifically stated otherwise.
- Missing values will not be imputed, unless specifically stated otherwise. A 'missing' category will be included for any parameter for which information is missing. This will ensure that the population size totals are consistent across different parameters.

8.1.3. Triplicate Readings

For vital signs data only, where triplicate readings are taken, the median of triplicate readings will replace the separate individual triplicate readings in all calculations.

For 12-lead ECG data only, where triplicate readings are taken, the mean of triplicate readings will replace the separate individual triplicate readings in all calculations.

In case of incomplete triplicate readings (eg, only 2 out of 3 readings were recorded), the mean and/or medians will be calculated, as appropriate, based on the number of readings available.

8.1.4. Repeat and Unscheduled Readings

For vital signs and 12-lead ECG data only, any predose value recorded in addition to the original value or a postdose value recorded within 15 minutes of the original value will be defined as a repeat value; any postdose value recorded more than 15 minutes after the original value will be defined as an unscheduled value. For all other data types (eg, laboratory parameters), any value recorded in addition to the original value will be defined as an unscheduled value.

The original value will be replaced by the last associated repeat value in all calculations, with the exception of the 12-lead ECG outlier analysis (see Section 8.6.4).

As unscheduled values are not associated with any scheduled timepoint, they will be excluded from all calculations, with the exception of the baseline derivation (see Section 8.1.5) and 12-lead ECG outlier analysis (see Section 8.6.4).

8.1.5. Definitions of Baseline and Change from Baseline

The baseline will be defined as the last value recorded prior to the first dose. If the date/time of the value is incomplete or missing, it will be excluded from the baseline calculation, unless the incomplete date/time indicates the value was recorded prior to the first dose.

Individual changes from baseline will be calculated by subtracting the individual subject's baseline value from the value at the postdose timepoint.

The summary statistics for change from baseline will be derived from individual subjects' values (eg, mean change from baseline will be the mean of the individual changes from baseline for all subjects, rather than difference between the mean value at the postdose timepoint and mean value at baseline).

See Section 8.1.4 for more detail on handling repeat and unscheduled readings in the calculations. See Section 8.1.3 for more detail on handling of triplicate readings in the calculations.

8.2. Subject Disposition and Population Assignment

Subject disposition and population assignment will be listed.

A summary table by treatment will be provided, based on the safety population.

8.3. Screening Demographics

The screening demographics including age, sex, race, ethnicity, height, body weight, and body mass index will be listed.

A summary table by treatment will be provided, based on the safety population.

8.4. Prior and Concomitant Medication

Prior medication will be defined as medication that ends prior to the first dose. Concomitant medication will be defined as medication that starts during or after the first dose or starts but does not end prior to the first dose.

Prior and concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) Global, Format B3, Version March 2021 (or later if a new version is issued during the study; see the data management plan [DMP] for more details). Prior and concomitant medications will be listed.

8.5. Pharmacokinetic Assessments

8.5.1. Pharmacokinetic Analysis

The following PK parameters will be determined where possible from the plasma and urine concentrations of NST-6179 using noncompartmental methods in validated software program Phoenix WinNonlin (Certara, Version 8.1 or higher):

Part A - SAD

Parameter	Units[a]	Definition
Plasma PK Parameters		
$AUC_{0\text{-}tlast}$	h*ng/mL	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration (t_{last})[b]
$AUC_{0\text{-}\infty}$	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity[c]
$AUC_{0\text{-}24}$	h*ng/mL	area under the concentration-time curve from time 0 to 24 hours postdose
$AUC_{0\text{-}72}$	h*ng/mL	area under the concentration-time curve from time 0 to 72 hours postdose (for calculation of CL_R)
$\%AUC_{extrap}$	%	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity
C_{max}	ng/mL	maximum observed concentration
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance
V_z/F	L	apparent volume of distribution during the terminal phase
$DAUC_{0\text{-}tlast}$	h*ng/mL/mg	$AUC_{0\text{-}tlast}$ normalised by dose administered[d]
$DAUC_{0\text{-}\infty}$	h*ng/mL/mg	$AUC_{0\text{-}\infty}$ normalised by dose administered[d]
DC_{max}	ng/mL/mg	C_{max} normalised by dose administered[d]
Urine PK Parameters		
$Ae_{t1\text{-}t2}$	mg	amount of the dose administered recovered over the time interval t1 to t2, calculated for each interval from the start of the collection interval and from the start of the dosing interval (time 0)
$fe_{t1\text{-}t2}$	%	percentage of the dose administered recovered over the time interval t1 to t2, calculated for each interval from the start of the collection interval and from the start of the dosing interval (time 0)
CL_R	L/h	renal clearance, calculated as $Ae_{0\text{-}72}/AUC_{0\text{-}72}$ or other appropriate collection interval

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration
[d] Calculated by dividing the parameter by the dose (mg)

Part B - MAD

Parameter	Units[a]	Definition
Plasma PK Parameters		
$AUC_{0-\tau}$	h*ng/mL	area under the concentration-time curve over a dosing interval, may be referred to AUC_{0-24} for Day 1[b]
$AUC_{0-\infty}$	h*ng/mL	area under the concentration-time curve from time 0 extrapolated to infinity[c] (Day 1 only)
$\%AUC_{extrap}$	%	percentage of area under the concentration-time curve due to extrapolation from the last quantifiable concentration to infinity (Day 1 only)
C_{max}	ng/mL	maximum observed concentration
C_{min}	ng/mL	minimum observed concentration in a dosing interval (Day 14 only)
t_{max}	h	time of the maximum observed concentration
t_{last}	h	time of the last quantifiable concentration
$t_{1/2}$	h	apparent terminal elimination half-life
CL/F	L/h	apparent total clearance
V_z/F	L	apparent volume of distribution during the terminal phase
$RA_{AUC0-\tau}$	NA	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	NA	observed accumulation ratio based on C_{max}
$DAUC_{0-\tau}$	h*ng/mL/mg	$AUC_{0-\tau}$ normalised by dose administered[d]
$DAUC_{0-\infty}$	h*ng/mL/mg	$AUC_{0-\infty}$ normalised by dose administered[d] (Day 1 only)
DC_{max}	ng/mL/mg	C_{max} normalised by dose administered[d]
Urine PK Parameters		
Ae_{t1-t2}	mg	amount of the dose administered recovered over the time interval t1 to t2, calculated for each interval from the start of the collection interval and from the start of the dosing interval (time 0)
fe_{t1-t2}	%	percentage of the dose administered recovered over the time interval t1 to t2, calculated for each interval from the start of the collection interval and from the start of the dosing interval (time 0)
CL_R	L/h	renal clearance, calculated as Ae/AUC using matched collection intervals; at steady state, collection intervals will be matched to the dosing interval

[a] Units are based on concentration units (provided by the bioanalytical lab or preferred units for presentation of PK parameters) and dose units used in the study.
[b] The AUC will be calculated using the linear trapezoidal rule for increasing concentrations and the logarithmic rule for decreasing concentrations (linear up/log down rule).
[c] Based on the last observed quantifiable concentration
[d] Calculated by dividing the parameter by the dose (mg)

Additional PK parameters may be determined where appropriate.

The PK analysis will be carried out where possible using actual dose administered (mg) and actual postdose blood sampling times. If an actual time is missing, the sample concentration result will be treated as missing unless there is scientific justification to include the result using the nominal time.

The parameters C_{max}, C_{min}, and t_{max} will be obtained directly from the concentration-time profiles. If C_{max} occurs at more than 1 timepoint, t_{max} will be assigned to the first occurrence of C_{max}.

The accumulation ratio(s) ($RA_{AUC0-\tau}$ and RA_{Cmax}) will be calculated as follows:

$RA_{AUC0-\tau}$ = $AUC_{0-\tau}$ profile day 14 / $AUC_{0-\tau}$ profile day 1

RA_{Cmax} = C_{max} profile day 14 / C_{max} profile day 1

The parameter $AUC_{0-tlast}$ or other common partial area may be used to determine $RA_{AUC0-\tau}$ if $AUC_{0-\tau}$ cannot be reliably calculated for the majority of subjects.

8.5.1.1. Criteria for the Calculation of Apparent Terminal Elimination Rate Constant and Half-life

The start of the terminal elimination phase for each subject will be defined by visual inspection and generally will be the first point at which there is no systematic deviation from the log-linear decline in concentrations.

The apparent terminal elimination rate constant (λ_z) will only be calculated when a reliable estimate can be obtained using at least 3 data points, preferably not including C_{max}, and the adjusted coefficient for determination of exponential fit (R^2-adj) of the regression line is ≥0.7. Parameters requiring λ_z for their calculation (eg, $AUC_{0-\infty}$, $t_{1/2}$, CL/F, and V_z/F) will only be calculated if the R^2-adj value of the regression line is ≥0.7.

The following regression-related diagnostic PK parameters will be determined, when possible:

Parameter	Units	Definition
λ_z	1/h	apparent terminal elimination rate constant
λ_z Upper	h	end of exponential fit
λ_z Lower	h	start of exponential fit
λ_z N	NA	number of data points included in the log-linear regression
λ_z Span Ratio	NA	time period over which λ_z was determined as a ratio of $t_{1/2}$
R^2-adj	NA	adjusted coefficient for determination of exponential fit

Where possible, the span of time used in the determination of λ_z (ie, the difference between λ_z Upper and λ_z Lower) should be ≥2 half-lives. If the λ_z Span Ratio is <2 half-lives, the robustness of the $t_{1/2}$ values will be discussed in the CSR.

8.5.1.2. Criteria for Calculation and Reporting of Area Under the Concentration-time Curve

The minimum requirement for the calculation of AUC will be the inclusion of at least 3 consecutive concentrations above the lower limit of quantification. If there are only 3 consecutive concentrations, at least 1 should follow C_{max}.

If the extrapolated area is >30%, $AUC_{0-\infty}$ (and derived parameters) may be excluded from the summary statistics and statistical analysis at the discretion of the sponsor or pharmacokineticist.

If $AUC_{0-\infty}$ cannot be determined reliably for all subjects and/or dose levels, an alternative AUC measure, such as AUC to a fixed timepoint or $AUC_{0-tlast}$, may be used in the statistical analysis of dose proportionality.

8.5.1.3. Calculation of NST-6179 Urinary Parameters

The amount of the dose administered recovered (Ae) in urine as NST-6179 for each urine collection interval (t_1-t_2) will be calculated as the product of urine analyte concentration and urine volume. Where only urine sample weight is supplied, a specific gravity of 1 g/mL will be assumed, and it will be considered equivalent to urine volume.

Cumulative $Ae_{0-x\ h}$ will be calculated by summing the Ae_{t1-t2} values over the 0-x h interval, where x = the end of the last collection time interval.

The percentage of the dose administered recovered over the time interval t1 to t2 (fe_{t1-t2}) as NST-6179 will be calculated for each urine collection interval as follows:

$$fe_{t1-t2} = (Ae_{t1-t2} / dose) \times 100$$

Cumulative $fe_{0-x\ h}$ will be calculated by summing the fe_{t1-t2} values over the 0-x h period in the same manner as cumulative $Ae_{0-x\ h}$.

Renal clearance (CL_R) will be calculated over 0-t2 according to the following formula, where cumulative Ae and AUC are calculable to the same end time (t2):

$$CL_R = Ae_{0-t2} / AUC_{0-t2}$$

Alternatively, $AUC_{0-\infty}$ may be used if urinary excretion of the dose is considered to be complete and $AUC_{0-\infty}$ is well characterised.

8.5.1.4. Criteria for Handling Concentration Below the Limit of Quantification or Missing Concentrations for Pharmacokinetic Analysis

Plasma concentrations below the limit of quantification (BLQ) will be assigned a value of 0 before the first measurable concentration and thereafter BLQ concentrations will be treated as missing. The following rules apply to the specific situations defined below:

- If an entire concentration-time profile is BLQ, it will be excluded from PK analysis.

- Where 2 or more consecutive concentrations are BLQ at the end of a profile, the profile will be deemed to have terminated and any further quantifiable concentrations will be set to missing for the calculation of the PK parameters, unless they are considered to be a true characteristic of the profile of the drug.

- If a Day 1 predose plasma concentration is missing, it will be set to 0 by default within Phoenix WinNonlin. If a profile day 14 predose concentration is missing, it will be set to Cmin by default within Phoenix WinNonlin.

Urine concentrations that are BLQ will be set to 0 for the calculation of Ae_{t1-t2}.

8.5.1.5. Treatment of Outliers in Pharmacokinetic Analysis

If a value is considered to be anomalous due to being inconsistent with the expected PK profile, it may be appropriate to exclude the value from the PK analysis. However, the exclusion of any data must have strong justification and will be documented in the CSR.

Any quantifiable predose concentration value on Day 1 will be considered anomalous and set to missing for the PK analysis. This will be set to 0 by default in Phoenix WinNonlin.

8.5.2. Presentation of Pharmacokinetic Data

All PK concentrations and parameters will be listed.

Summary tables, arithmetic mean (+ standard deviation [SD]) figures, overlaying individual figures, and individual figures by treatment and time postdose will be provided for plasma PK concentrations. All figures will be produced on both linear-linear and linear-logarithmic scales, with the exception of figures across all days, which will be produced on the linear-linear scale only. The +SD bars will only be displayed on the linear-linear scale.

Summary tables by treatment will be provided for all PK parameters, with the exception of diagnostic regression-related PK parameters. Separate summary tables by treatment and time interval will be provided for excretion parameters and cumulative excretion parameters.

A subject may be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2 times the median t_{max}.

If the actual time of sample collection deviates from the nominal time by more than ±10%, the plasma concentration will be flagged in the listings. Individual concentrations deemed to be anomalous will be flagged in the listings.

For plasma concentration data the following rules will apply:

- Values that are BLQ will be set to 0 for the calculation of summary statistics.

- Arithmetic mean or median values that are BLQ will be presented as 0.

Urine concentration data will be listed only.

For PK parameters the following rule will apply:

- Geometric mean and coefficient of variation will not be calculated for discrete time parameters (ie, t_{max} or t_{last}).

8.5.3. Pharmacokinetic Statistical Methodology

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} on profile day 1 for Part A; $AUC_{0\text{-}\infty}$ and C_{max} on profile day 1 and $AUC_{0\text{-}\tau}$ and C_{max} on profile day 14 for Part B (only for plasma NST-6179).

The hypothesis testing will be 2-sided and carried out on 0.05 significance level.

The PK parameters will be analysed using a power model[4] that will have the following form:

$$parameter = intercept \times dose^{\wedge}slope \times random\ error$$

Using the natural log (ln) transformation,[5] a power model can be expressed as a linear regression equation:

$$ln(parameter) = intercept + slope \times ln(dose) + random\ error$$

For dose proportionality, the slope of the regression line is equal to 1; for dose independence, it is equal to 0.

For each PK parameter separately, a pooled estimate (across all doses) of slope, corresponding 95% confidence interval (CI), and between-subject CV will be calculated. Figures (on the logarithmic-logarithmic scale) containing individual values, power model line (95% CI), and dose proportionality line (defined as the power model line with slope of 1) will be created for each PK parameter. Additionally, figures (on the logarithmic-linear scale) containing individual values and geometric means will be created for each corresponding PK parameter normalised by dose administered.

The lack of fit test will be conducted for the statistical assessment of linearity assumption, and thus appropriateness of a power model. The lack of fit model will be the same as the power model fitted, but with dose included as additional fixed effect. The statistical assessment will rule the linearity assumption acceptable if the diagnostic plots appear reasonable and the lack of fit 2-sided p-value >0.05 (dose effect is not significant at the 0.05 level of significance). The assessment of linearity assumption may also occur via visual examination of the figures by the pharmacokineticist. This assessment may override the statistical assessment; where this occurs, it will be detailed in the CSR.

It will be concluded that PK parameter is dose proportional for the dose range studied if the assumption of linearity is ruled acceptable and the 95% CI for the slope spans 1.

If the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be ln-transformed and analysed using an analysis of variance (ANOVA) model.[6] The model will include dose as a factor.

For each PK parameter separately, the geometric least squares mean (GLSM) for each dose, p-values for the overall, and pairwise dose comparisons will be calculated. Residual plots will be produced to assess the adequacy of the model(s) fitted.

Examples of the SAS code that will be used are as follows:

Power Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday fast paramn param;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output solutionf = <data out>;
run;
```

Power Model Analysis (Between-subject Variability)

```
proc mixed data = <data in> covtest alpha = 0.05;
 by parcat1n parcat1 pkday fast paramn param;
 class ldose;
 model lpk = ldose / cl residual ddfm = kr2;
 ods output covparms = <data out>;
run;
(Note: Pooled Geometric CV (%) = 100*(sqrt(exp(estimate)-1)))
```

Power Model Analysis (Lack of Fit Test)

```
proc mixed data = <data in>;
 by parcat1n parcat1 pkday fast paramn param;
 class dose;
 model lpk = ldose dose / htype = 1 ddfm = kr2;
 ods output tests1 = <data out>;
run;
```

ANOVA Model Analysis

```
proc mixed data = <data in> alpha = 0.05;
 by parcat1n parcat1 pkday fast paramn param;
 class dose;
 model ldnpk = dose / cl residual ddfm = kr2;
 lsmeans dose / cl pdiff;
 ods output lsmeans = <data out>;
 ods output diffs = <data out>;
 ods output tests3 = <data out>;
run;
```

8.6. Safety and Tolerability Assessments

8.6.1. Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 24.0 (or higher if a new version is issued during the study; see the DMP for more details).

A treatment-emergent adverse event (TEAE) will be defined as an AE that starts during or after the first dose, or starts prior to the first dose and increases in severity after the first dose.

A treatment-related TEAE will be defined as a TEAE with a relationship of related to the study treatment, as determined by the investigator.

All AEs will be listed. In addition to the data recorded in the database, the listings will include derived onset time and duration. Onset time will be calculated from the time of the last associated dose for TEAEs only. Where the last associated dose is referring to the last dose received prior to the start of a TEAE.

The frequency of subjects with TEAEs and the number of TEAEs will be summarised for the following categories:

- TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- TEAEs by severity and treatment
- Treatment-related TEAEs (overall, serious, leading to discontinuation, and leading to death) by treatment
- Treatment-related TEAEs by severity and treatment

The frequency of subjects will be summarised separately for TEAEs and treatment-related TEAEs by the following:

- System organ class, preferred term, and treatment
- Preferred term and treatment

For the AE data the following rules will apply:

- For the derivation of treatment-emergent status (applicable to all AEs): If the start date/time of an AE is incomplete or missing, an AE will be assumed to not be a TEAE, unless the incomplete start date/time or the end date/time indicates an AE started after the first dose.
- For the derivation of treatment-related status (applicable to TEAEs only): If the study treatment relationship for a TEAE is missing, a TEAE will be assumed to not be a treatment-related TEAE.
- For the derivation of onset time (applicable to TEAEs only): If the start date/time of a TEAE is missing, onset time will not be calculated. If the start date/time of a TEAE is incomplete, where possible, the minimum possible onset time will be calculated and presented in '≥DD:HH:MM' format (eg, if the date/time of the last associated dose is 01MAY2019/08:00 and recorded start date/time of a TEAE is 03MAY2019, then the minimum possible onset time will be calculated by assuming a TEAE started at the first hour and minute of 03MAY2019 [03MAY2019/00:00], thus will be presented as onset time ≥01:16:00 in the listing). If the start date of a TEAE is the same as the date of the last associated dose but the start time of a TEAE is missing, an onset time will be presented as '≥00:00:01'. Any clock changes will be accounted for in the derivation.

- For the derivation of duration (applicable to all AEs): If the end date/time of an AE is missing, duration will not be calculated. If the start or end date/time of an AE is incomplete, where possible, the maximum possible duration will be calculated and presented in '≤DD:HH:MM' format (eg, if the start of an AE date/time is 01MAY2019/08:00 and its recorded end date/time is 03MAY2019, then the maximum possible duration will be calculated by assuming an AE ended at the last hour and minute of 03MAY2019 [03MAY2019/23:59], thus will be presented as duration ≤02:15:59 in the listing). Any clock changes will be accounted for in the derivation.

- For the calculation of TEAE summary statistics: If the severity of a TEAE is missing, that TEAE will be counted under the 'missing' category.

- For the calculation of TEAE summary statistics: If a subject experienced multiple TEAEs with the same preferred term for the same treatment, this will be counted as 1 TEAE for that treatment under the maximum severity recorded.

8.6.2. Clinical Laboratory Parameters

All clinical laboratory parameters will be listed; any value outside the clinical reference range will be flagged. Separate listings will be provided for any parameter for which there is any individual subject value outside the respective clinical reference range.

Summary tables and boxplots by treatment and timepoint will be provided for clinical chemistry, haematology, and coagulation.

Values recorded as <x, ≤x, >x, or ≥x will be displayed in the listings as recorded. For the derivation of listing flags, all calculations, and presentation in the figures, <x and ≤x values will be set to half of x, whereas >x and ≥x values will be set to x.

8.6.3. Vital Signs Parameters

All vital signs parameters and their changes from baseline will be listed, as applicable; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by treatment and timepoint will be provided for all vital signs parameters and their changes from baseline, as applicable.

8.6.4. 12-lead Electrocardiogram Parameters

All 12-lead ECG parameters and their changes from baseline will be listed; any value outside the clinical reference range will be flagged.

Summary tables and boxplots by treatment and timepoint will be provided for all 12-lead ECG parameters and their changes from baseline.

An outlier analysis will be performed for QT interval corrected for heart rate using Bazett's formula (QTcB) and QT interval corrected for heart rate using Fridericia's formula (QTcF). The analysis will include all individual original, repeat, and unscheduled postdose values.

The maximum postdose values will be summarised by treatment according to the following categories:

- ≤450 ms
- >450 and ≤480 ms (all instances flagged in the listing)
- >480 and ≤500 ms (all instances flagged in the listing)
- >500 ms (all instances flagged in the listing)

The maximum increases from baseline will be summarised by treatment according to the following categories:

- ≤30 ms
- >30 and ≤60 ms (all instances flagged in the listing)
- >60 ms (all instances flagged in the listing)

8.6.5. Other Assessments

Medical history will not be listed.

The continuous 12-lead ECG data will be recorded for this study, but if it is analysed in the future, it will be reported separately.

All other safety and tolerability assessments not detailed in the above sections will be listed only.

8.6.6. Safety and Tolerability Statistical Methodology

No inferential statistical analyses are planned.

9. INTERIM ANALYSES

No formal interim analyses are planned for this study.

10. SIGNIFICANT CHANGES FROM THE PROTOCOL-SPECIFIED ANALYSES

There were no significant changes from the protocol-specified analyses.

11. REFERENCES

1. ICH. ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (E3). 30 November 1995.
2. ICH. ICH Harmonised Tripartite Guideline: General considerations for clinical trials (E8). 17 July 1997.

3. ICH. ICH Harmonised Tripartite Guideline: Statistical principles for clinical trials (E9). 5 February 1998.

4. Gough K, Hutchinson M, Keene O, et al. Assessment of dose proportionality: report from the statisticians in the pharmaceutical industry/Pharmacokinetics UK Joint Working Party. *Drug Inf J*. 1995;29(3):1039-1048.

5. Keene ON. The log transformation is special. *Stat Med*. 1995;14(8):811-819.

6. Brown H, Prescott R. *Applied Mixed Models in Medicine*. Chichester: John Wiley & Sons, 1999.

12. APPENDICES

Appendix 1: Document History

Status and Version	Date of Change	Summary/Reason for Changes
Final Version 1.0	NA	NA; the first version.

NA = not applicable