Protocol

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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SPONSOR APPROVAL

I have read the protocol and approve it:

Electronically signed below — 13 Jul 2022

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INVESTIGATOR AGREEMENT

I have read the protocol and agree to conduct the study as described herein.

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RATIONALE FOR PROTOCOL AMENDMENT

In order to increase the overall extent of exposure without exceeding the pharmacokinetic (PK) stopping criteria, twice daily (BID) dosing is planned to be investigated within Part B of the study. The dose selected per dosing occasion in Part B will not exceed a single dose shown to be safe and well tolerated in Part A.

Due to the rapid elimination of NST-6179, no accumulation is expected following BID dosing. However, to enhance safety, a requirement for sentinel dosing in Part B has been added for BID dose regimens where the total daily dose exceeds the highest single dose evaluated in Part A. Relevant clinical PK and clinical safety data from previous cohorts in this study have been added to support these changes.

Clarifications have been added regarding dose administration in the fasted state for a BID dosing regimen and the schedule of assessments for Part B has been updated to include options for BID dosing.

Additionally, the option for the collection of a pharmacogenomics (PGx) blood sample from each subject has been added along with an associated exploratory objective and endpoint. Nonclinical studies show that the principal route of the hepatic metabolism of NST-6179 *in vitro* is catalysed by cytochrome P450 (CYP)2C9 which is a polymorphic enzyme. Blood samples for PGx analyses will be collected to explore the contribution of common gene variants, such as CYP2C9, to the variability in PK profiles between subjects *in vivo*. The optional collection of the PGx blood sample will apply to all subjects in the study, in the case that subjects have already been dosed and/or discharged they will be contacted and asked if they would be willing to return to the CRU and consent to have this sample collected.

The major and minor changes implemented in this amendment are summarised in Appendix 7.

STUDY IDENTIFICATION

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SYNOPSIS

Study Title

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

Objectives

The primary objectives of the study are:

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

The secondary objective of the study is:

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

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.
- to evaluate the effect of pharmacogenomics on the PK of NST-6179 in healthy male and female subjects.

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.

Part A will comprise a double-blind, single-ascending dose, sequential-group design.

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.

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.

Part B will comprise a double-blind, multiple-ascending dose, sequential-group study design. 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.

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.

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. There will be a minimum of 14 days between dose escalations for each group. The dose selected on each dosing occasion in Part B will not exceed a single dose shown to be safe and well tolerated in Part A. Sentinel dosing will be implemented for any groups in Part B for which the total daily dose exceeds the highest single dose evaluated in Part A (eg, 1000 mg BID for which the total daily dose of 2000 mg has not been administered as a single dose in Part A), whereby an initial cohort of 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining cohort of 8 subjects (7 active and 1 placebo) will be dosed after at least 72 hours.

Number of Subjects

Part A: it is planned to study 48 subjects in 6 groups (Groups A1 to A6). If additional groups are studied, up to 72 subjects will be studied in 9 groups (Groups A1 to A9).

Part B: it is planned to study 40 subjects in 4 groups (Groups B1 to B4). If additional groups are studied, up to 70 subjects will be studied in 7 groups (Groups B1 to B7).

Diagnosis and Main Criteria for Inclusion

In Part A and Part B, healthy male and female subjects aged between 18 and 65 years (inclusive) with a body mass index between 18.0 and 32.0 kg/m^2 (inclusive).

Investigational Medicinal Products, Dose, and Mode of Administration

10 mg and 100 mg NST-6179 capsules.

Proposed dose levels for Part A: 50 mg for Group A1; subsequent dose levels are to be determined 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.

Proposed dose levels for Part B: the dose levels, dosing frequency, and dosing duration will be decided, in consultation with the sponsor, on the basis of data from Part A of the study or earlier groups in Part B. The dose of NST-6179 per dosing occasion in Part B will not exceed a single dose shown to be safe and well tolerated in Part A.

Administration route: oral.

Reference Product and Mode of Administration for Part A and Part B

Reference product: placebo capsules.

Administration route: oral.

Duration of Subject Participation in the Study

Part A

Planned screening duration: approximately 4 weeks.

Planned study duration (screening to follow-up): approximately 6 weeks.

Part B

Planned screening duration: approximately 4 weeks.

Planned study duration (screening to follow-up): approximately 8 weeks.

Endpoints

Pharmacokinetics

Blood and urine samples will be collected for the analysis of plasma and urinary concentrations of NST-6179. Pharmacokinetic parameters will be derived by non-compartmental analysis. For Part A, the PK parameters will include area under the concentration-time curve (AUC) from time zero to infinity ($AUC_{0-\infty}$), area under the concentration-time curve from time zero to infinity normalised by dose ($AUC_{0-\infty}$/dose), area under the concentration-time curve from time zero to the time of the last quantifiable concentration ($AUC_{0-tlast}$), area under the concentration-time curve from time zero to the time of the last quantifiable concentration normalised by dose ($AUC_{0-tlast}$/dose), maximum observed concentration (C_{max}), C_{max} normalised by dose (C_{max}/dose), time of the maximum observed concentration (t_{max}), apparent terminal elimination half-life ($t_{½}$), apparent total clearance (CL/F), apparent volume of distribution (V_z/F), amount of drug excreted (A_e), percentage of dose excreted unchanged (f_e), and renal clearance (CL_R). In Part B, the PK parameters will include: area under the concentration-time curve over a dosing interval ($AUC_{0-\tau}$), AUC time zero to 24 hours postdose (AUC_{0-24}, derived as $AUC_{0-\tau}$*2 for a BID dosing regimen at steady state), area under the concentration-time curve over a dosing interval normalised by dose ($AUC_{0-\tau}$/dose), $AUC_{0-\infty}$ (Day 1 only), $AUC_{0-\infty}$/dose (Day 1 only), C_{max}, C_{max}/dose, t_{max}, $t_{½}$, CL/F, V_z/F, minimum observed concentration (C_{min}), observed accumulation ratio based on $AUC_{0-\tau}$ ($RA_{AUC0-\tau}$), and observed accumulation ratio based on C_{max} (RA_{Cmax}). Other PK parameters may also be added.

Safety

Adverse events (AEs), clinical laboratory evaluations (haematology, clinical chemistry, coagulation, and urinalysis), 12-lead electrocardiogram (ECG) parameters, vital signs measurements, and physical examinations.

Exploratory

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

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

Optional pharmacogenomics samples will be collected from subjects that consent, for the determination of common gene variants (ie, cytochrome P450 2C9), that may have an effect on the PK of NST-6179.

Statistical Methods

Pharmacokinetics

Non-compartmental PK analysis will be performed on individual plasma and urine concentration data, using commercial software such as Phoenix® WinNonlin®.

The PK concentrations and parameters will be listed and summarised using descriptive statistics.

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} on Day 1 in Part A and $AUC_{0\text{-}\tau}$ and C_{max} on Day 14 in Part B. The PK parameters will be analysed using a power model. However, if the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be natural log-transformed and analysed using an analysis of variance model.

Safety

Safety parameters will be listed and summarised using descriptive statistics. No formal statistical analysis of safety data is planned.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
A_e	amount of drug excreted
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AM	morning
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
$AUC_{0-\infty}$	area under the concentration-time curve from time zero to infinity
AUC_{0-24}	area under the concentration-time curve from time zero to 24 hours postdose
$AUC_{0-tlast}$	area under the concentration-time curve from time zero to the time of the last quantifiable concentration
$AUC_{0-\tau}$	area under the concentration-time curve over a dosing interval
BID	twice daily
BMI	body mass index
bpm	beats per minute
CFR	Code of Federal Regulations
CL/F	apparent total clearance
CL_R	renal clearance
C_{max}	maximum observed concentration
C_{min}	minimum observed concentration
COVID-19	Coronavirus Disease 2019
CRO	contract research organisation
CRU	Clinical Research Unit
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
f_e	percentage of drug excreted
FO	fish oil
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HED	human equivalent dose
hERG	human ether-à-go-go-related gene
ICF	informed consent form

ICH	International Council for/Conference on Harmonisation
IFALD	intestinal failure-associated liver disease
IMP	investigational medicinal product
IRB	institutional review board
IUD	intrauterine device
MCFA	medium chain fatty acid
MHRA	Medicines and Healthcare products Regulatory Agency
MTD	maximum tolerated dose
NOAEL	no-observed-adverse-effect level
PGx	pharmacogenomic
PK	pharmacokinetic(s)
PM	evening
PNALD	parenteral nutrition associated liver disease
QD	once daily
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's method
$RA_{AUC0-\tau}$	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	observed accumulation ratio based on C_{max}
SAE	serious adverse event
$t_{½}$	apparent terminal elimination half-life
TK	toxicokinetic(s)
t_{max}	time of the maximum observed concentration
TMF	trial master file
TPN	total parenteral nutrition
ULN	upper limit of normal
V_z/F	apparent volume of distribution

1. INTRODUCTION

1.1. Overview

NorthSea Therapeutics is developing NST-6179, a novel, orally administered, fully-synthetic medium chain fatty acid (MCFA) analogue for the treatment of intestinal failure-associated liver disease (IFALD), also known as parenteral nutrition associated liver disease (PNALD), and other potential indications. There are likely to be multiple mechanisms that contribute to the observed pharmacodynamic (PD) effects of NST-6179. NST-6179 is structurally based on a MCFA to maximise both portal vein uptake and passive absorption but is modified to resist rapid metabolism as an energy source. This combination of attributes may provide targeted therapy for the treatment of IFALD.

Total parenteral nutrition (TPN) is a lifesaving therapy for individuals with intestinal failure caused by insufficient bowel length or function. Long-term TPN treatment is limited by potentially serious complications, including liver disease. In the United States, it is estimated that around 40,000 to 45,000 patients require long term TPN, of which approximately 20% progress to IFALD. The incidence of IFALD in patients receiving long-term TPN varies widely depending on the age group, from 15% to 40% in adults and 40% to 60% in infants and neonates. Approximately 22% of deaths in patients on long-term TPN are attributed to IFALD. Intestinal failure-associated liver disease development is characterised initially by intrahepatic cholestasis, and then by progressive fibrosis and ultimately cirrhosis over the course of several months. Current strategies to treat IFALD include lipid restriction, which carries the risk of essential fatty acid deficiency, or transition to high volumes of fish oil (FO) lipid emulsion per day based on the weight of an average adult. There is a population of patients who continue to develop IFALD despite having been weaned off and it has also been demonstrated that a significant percentage of patients fail therapy. As such, there is a need for alternative treatments for patients who may benefit from lower dose enterally administered therapies.

This study will be the first time NST-6179 has been administered to humans.

1.2. Summary of Non-clinical Pharmacology

Three *in vivo* pharmacology studies were conducted.

Non-clinical efficacy studies demonstrated that in murine models of IFALD, orally administered NST-6179 was absorbed enterally and led to preservation of normal liver architecture, as compared to vehicle groups, which developed significant steatosis. Hepatic lipidomic analysis confirmed a decrease in hepatic triglycerides (-55%) and cholesteryl ester, the main hepatic cholesterol storage depot. The results demonstrated that NST-6179 confers hepatoprotective properties in a murine model of TPN-induced liver injury. An additional study where the same model was used to investigate co-administration of NST-6179 with either Intralipid (Soybean [SO] oil) or Omegaven® (FO) demonstrated that the combination did not give rise to any detrimental effects in this model and as such suggests that NST-6179 could be safely administered in combination with existing lipid emulsion therapy. Importantly, significant down-regulation of key genes regulating hepatic inflammation and fibrosis were also observed in a mouse model of fibrosing steatohepatitis in response to

over-nutrition. These results suggest NST-6179 as a potential treatment for patients with IFALD or who are at risk of developing IFALD.

1.3. Summary of Safety Pharmacology

Four safety pharmacology studies were completed (all conducted to Good Laboratory Practice [GLP]).

In an *in vitro* human ether-à-go-go-related gene (hERG) assay, NST-6179 had no significant effect on hERG tail current up to 75 μM (maximum concentration tested and limit of solubility).

Consistent with the findings from the hERG study, no effect on the cardiovascular parameters, electrocardiogram (ECG) parameters, or body temperature in the telemetered cynomolgus monkey were observed following NST-6179 at doses of 60 mg/kg, 175 mg/kg, and 500 mg/kg. It was therefore concluded that the no-observed-adverse-effect level (NOAEL) for NST-6179 in the monkey was 500 mg/kg due to no adverse findings on the cardiovascular system and on body temperature. In addition, no adverse findings were noted in the ECGs of monkeys treated for 28 days up to 350 mg/kg in the repeat-dose toxicity study (Section 1.4.2).

In an Irwin's test in the Han Wistar rat, a single dose of NST-6179 at 100 mg/kg and 245 mg/kg was not associated with any adverse (biologically significant) observations. Following single oral administration of 245 mg/kg NST-6179 in male rats, no adverse effects on the behavioural and physiological state of the rats were recorded; however, statistically significant reductions in locomotor activity and rearing were recorded at 2 hours postdose (not observed at the 100 mg/kg dose level). The NOAEL was considered to be 245 mg/kg, due to no adverse behavioural signs and the effects on locomotor activity and rearing were not considered to be adverse.

A single dose study was performed to determine the potential effects of NST-6179 following single oral administration on respiratory rate, tidal volume, and minute volume in the rat. In this study, NST-6179 was administered at doses of 100 mg/kg, 245 mg/kg, and 600 mg/kg. The vehicle control was corn oil. There were no adverse or biologically relevant effects on respiratory rate, tidal volume, or minute volume. In addition, no significant adverse clinical signs were recorded in either the vehicle or any of the NST-6179-treated groups. Based on these findings, the NOAEL was 600 mg/kg.

1.4. Summary of Toxicology

Repeat-dose toxicology studies were conducted in rats and cynomolgus monkeys given oral NST-6179 for 28 days followed by a 2-week recovery period.

1.4.1. 28-day Oral (Gavage) Administration Toxicity Study in the Rat Followed by a 2-week Recovery Period

NST-6179 was administered at doses of 100 mg/kg/day (low dose), 245 mg/kg/day (intermediate dose), and 600/450 mg/kg/day (high dose) to male and female rats. Animals in the high dose group were dosed 600 mg/kg/day on Days 1 and 2 of the dosing phase,

followed by a 2-day washout period and recommenced dosing at 450 mg/kg/day from Day 5 of the dosing phase. At a dose level of 600 mg/kg/day, there were observations of decreased activity on Days 1 and 2, and ataxia, shallow respiration, splayed gait, decreased body tone, vocalisation, piloerection, and partially closed eyes on Day 2. The severity of these findings resulted in the suspension of administration of 600 mg/kg/day and the high dose was reduced to 450 mg/kg/day. The vehicle control article in this study was corn oil. Assessment of toxicity was based on mortality, clinical and postdose observations, body weights, food consumption, ophthalmic observations, and clinical and anatomic pathology.

There were no clinical observations of concern following administration up to 450 mg/kg/day (reduced high dose) and there were no effects on body weights for female dose groups and no ophthalmic findings in animals of either sex administered NST-6179.

Daily oral gavage administration of 100 mg/kg/day, 245 mg/kg/day or 600/450 mg/kg/day NST-6179 was tolerated at 100 mg/kg/day and 245 mg/kg/day, with no notable clinical observations or postdose observations in either dose group and only a slight reduction in body weight for males and slightly increased food consumption for females administered 245 mg/kg/day.

Administration of 600 mg/kg/day NST-6179 resulted in the premature sacrifice of 1 female rat and the death of 1 female rat with a range of adverse clinical observations and initial weight loss and reduced food consumption were noted for males. Due to these findings, the dose of 600 mg/kg/day was reduced to 450 mg/kg/day for the remaining animals.

Once daily (QD) administration of 450 mg/kg/day also produced no in-life clinical observations, but males were observed with body weight losses. Findings related to an effect on the liver were observed in all NST-6179 dose groups and included diffuse hepatocyte hypertrophy and increased liver weights; these findings were observed in a dose-related manner and considered of a greater severity for males administered 600/450 mg/kg/day than animals administered lower doses of 100 mg/kg/day or 245 mg/kg/day, and correlated with increased alanine aminotransferase (ALT) activity across all dose levels, and alkaline phosphatase (ALP) activity at the 600/450-mg/kg/day dose level. Plasma drug levels were lower after repeated administration suggestive of metabolic induction.

A dose level of 450 mg/kg/day resulted in several findings, but no single finding was considered adverse. Full reversibility of all findings, including the liver findings following administration of 450 mg/kg/day, indicated that any mild effects at this or lower doses would be fully reversible.

Based on these findings, the NOAEL is 450 mg/kg/day. This dose level corresponded to mean maximum observed plasma concentration (C_{max}) values of 95,400 ng/mL (males) and 66,500 ng/mL (females), and mean area under the plasma concentration-time curve from time zero to 24 hours postdose (AUC_{0-24}) values of 1,160,000 ng.h/mL (males) and 1,030,000 ng.h/mL (females; Section 1.5.1).

1.4.2. 4-week Oral (Gavage) Toxicity and Toxicokinetic Study in the Monkey with a 2-week Recovery Period

NST-6179 was administered at doses of 60 mg/kg/day (low dose), 175 mg/kg/day (intermediate dose), and 500/350 mg/kg/day (high dose) to male and female monkeys. Animals were dosed via oral gavage QD for 28 days at a volume of 5 mL/kg. Animals in the high dose group were dosed at 500 mg/kg/day from Day 1 to Day 6 of the dosing phase, followed by a 30-day washout period and recommenced dosing at 350 mg/kg/day for 28 days. At a dose level of 500 mg/kg/day, there were observations of decreased activity, ataxia, semi-closed eyes, hunched posture, and vomiting. The severity of these findings and 2 mortalities resulted in suspension of administration of 500 mg/kg/day, and the high dose was reduced to 350 mg/kg/day. The vehicle control article in this study was corn oil. Assessment of toxicity was based on mortality, clinical and postdose observations, body weights, food consumption, ophthalmic observations, ECG, and clinical and anatomic pathology.

One male administered 500 mg/kg/day died following sedation due to the severity of clinical observations, which included a severe reduction in activity on Day 2 of dosing. One female administered 500 mg/kg/day was found dead on Day 7 of dosing. Observations noted for this animal on Day 6 of dosing included reduced activity. Administration of 500 mg/kg/day resulted in postdose observations of decreased activity, ataxia, semi-closed eyes, hunched posture, and vomiting. The severity of these findings and 2 mortalities resulted in suspension of administration of 500 mg/kg/day after 6 days, and the high-dose level was reduced to 350 mg/kg/day following a 30-day wash-out period. Administration of 350 mg/kg/day was only associated with transient, mild, and occasional postdose observations of decreased activity, hunched posture, and vomiting that diminished throughout the 28 days of dosing.

Administration of 350 mg/kg/day or 500 mg/kg/day resulted in increased body weight losses (up to 6.6% and 6.8% for males and females, respectively, at the 350-mg/kg/day dose level, and up to 5.9% and 5.2% for males and females, respectively, at the 500-mg/kg/day dose level) over the relevant dosing periods.

Clinical chemistry changes observed during Week 4 of the dosing phase included a slightly increased ALT for both sexes administered 350 mg/kg/day (10.6% and 24.1% for males and females, respectively) and males administered 175 mg/kg/day (14.9%). In addition, there was increased triglyceride levels for both sexes administered 350 mg/kg/day (84.2% and 21.3% for males and females, respectively) and females administered 175 mg/kg/day (12.9%) when compared with predose results. Triglyceride levels remained increased for animals administered 350 mg/kg/day (by 65.8% and 29.8% for males and females respectively when compared to predose values) at the end of the recovery phase, with ALT activity no longer increased for animals administered 350 mg/kg/day.

Daily oral gavage administration of NST-6179 at doses of 60 mg/kg/day, 175 mg/kg/day, or 350 mg/kg/day was well tolerated. Administration of 500 mg/kg/day was clearly not tolerated and resulted in the death of 1 male and 1 female, alongside severe adverse clinical observations of ataxia and decreased activity. Based on these findings, the NOAEL is considered to be 350 mg/kg/day. This dose level corresponded to mean C_{max} values of

145,000 ng/mL (males) and 59,100 ng/mL (females), and mean AUC_{0-24} values of 638,000 ng.h/mL (males) and 440,000 ng.h/mL (females).

1.4.3. 13-week Oral (Gavage) Toxicity and Toxicokinetic Study in the Monkey with a 2-week Recovery Period

This is an ongoing study objective to evaluate the toxicity and determine the toxicokinetics (TK) of NST-6179 when administered daily via oral gavage to the monkey for at least 13 weeks and to assess the reversibility or persistence of any effects after a 4-week recovery phase following doses of 60 mg/kg/day (low dose), 210 mg/kg/day (intermediate dose), and 350 mg/kg/day (high dose). The purpose of the study is to establish dose levels for future long term toxicity studies. A high dose level of 350 mg/kg/day was selected based on results from a 28-day repeat dose toxicity study (Covance study 8423746). An original high dose of 500 mg/kg/day was not tolerated, resulting in two early sacrifices and postdose observations of decreased activity, ataxia, semi-closed eyes, hunched posture, and vomiting. Following a 30-day washout period, a revised high dose level of 350 mg/kg/day was investigated over 28 days, which was tolerated with no mortality and transient mild and occasional postdose observations of decreased activity, hunched posture, and vomiting that diminished throughout the 28 days dosing. An intermediate dose level of 210 mg/kg/day was selected as an alternative high dose level for future studies if the selected high dose level was not tolerated. A low dose level of 60 mg/kg/day was expected to be tolerated in-life, with no postdose observations or body weight effect noted following 28 days repeat administration at this dose level during the previous toxicity study. The cynomolgus monkey was selected as the relevant species because of the similarity of monkeys to humans in species specific cross-reactivity of test article. An *in vitro* metabolism study (Covance study 8423743) indicated the cynomolgus monkey is the preferred choice of large animal species due to the presence of specific human metabolites, which are not present in other species.

Animals were dosed through Day 15 and NST-6179 was generally well tolerated with only mild spurious vomiting and an 8% decrease in bodyweight observed in the early stages, both of which returned to baseline status over time. No other relevant clinical findings have been observed to date at any dose level in either male or female monkeys. However, there were subsequently 2 events of note in the high-dose cohort (350 mg/kg/day). The first event occurred on Day 16 in which a single female monkey was found dead following dosing on Day 15. The decedent female appeared to be in general good health and displayed no clinical signs up through and postdose on Day 15. A macroscopic post-mortem examination showed no advanced autolysis at macroscopic post-mortem indicating the female died relatively soon prior to the post-mortem examination and at a timeframe when minimal drug concentrations levels would have been present. Microscopically, minimal alveolar/interstitial inflammation was noted, consistent with post aspiration effects but not the cause of mortality. The majority of tissues were well preserved with some autolysis noted in the gall bladder and gastrointestinal tract and to a lesser extent liver, pancreas, and salivary glands. No pre-existing comorbid conditions were identified as a potential cause of death. Clinical chemistry and urinalysis parameters were all normal or with minimal changes; none of which were felt to be clinically relevant. Assessment of the decedents ECG indicated minor anomalies considered not clinically relevant. As of this time, there is no clear indication of the cause of death but the examinations to date do not show any evidence of direct tissue injury or toxicity associated with NST-6179.

The second event occurred in the high-dose cohort (350 mg/kg/day) where a male monkey was found in a moribund condition 4 hours after dosing and euthanized on Day 87. This male was in good health throughout the study and prior to the last dose on Day 87. The investigation into the cause of death is still ongoing as well as an assessment of the remaining animals in the high-dose cohort. However, the initial assessment from the study pathologist determined that the microscopic findings were common background changes in this strain of monkey. However, there was moderate bilateral tubular nephropathy in the kidney which could be possibly related to the cause of death but will be confirmed with further investigations of this animal and the others in the cohort. No other clinical or laboratory findings revealed any clear signs of drug toxicity that would lead to death.

A review of the data from the prior 28-day repeat dose oral toxicity study in monkeys established 350 mg/kg/day was the NOAEL level (Covance 8423746). No clinical signs and symptoms or mortality were observed at this dose level. Importantly, dosing in the monkey 13-week study is now complete. NST-6179 was well tolerated in all other surviving male and female high dose monkeys. The low (60 mg/kg/day) and medium (210 mg/kg/day) doses were well-tolerated throughout the study period with no notable events. Both of the events in the high-dose group were at exposures far exceeding the exposure of the likely doses in the Phase 1 study. In addition, there was no clear drug-related effect with the first monkey and the second event occurred at a duration much longer than the maximal 14-day duration in the Phase 1 study.

1.4.4. 13-week Oral (Gavage) Administration Toxicity Study in the Mouse (Labcorp Study Number 8423764; ongoing)

A 13-week oral toxicity study in the mouse conducted under GLP is currently ongoing to determine dose level selection in a subsequent 2-year carcinogenicity study as well as evaluating TK at Day 1 and Day 90 of the study.

The maximum dose levels administered in the 13-week study were based on a previous mouse dose range finding study which was conducted to establish the maximum tolerated oral dose (MTD) in male and female mice. This study established an MTD of 250 mg/kg in female mice and an MTD of 200 mg/kg/day in male mice. These dose levels were tolerated for 21 days without mortality or adverse clinical signs. The selected low dose level of 70 mg/kg/day is the approximate effective dose. The intermediate dose levels in males and females represent the mid-point between 70 mg/kg/day and 200 mg/kg/day (males) and 250 mg/kg/day (females).

Group assignment is indicated in Table 1.

Table 1: Group Assignment in the 13-week Oral (Gavage) Administration Toxicity Study in the Mouse (Labcorp Study Number 8423764)

Group[a]	Subgroup	Dose Level[b] (mg/kg/day)	Dose Concentration[b] (mg/mL)	Number of Animals	
				Males	Females
1 (Control)	1 (Toxicity)	0	0	12	12
	2 (Toxicokinetic)	0	0	3	3
2 (Low)	1 (Toxicity)	70	7	12	-
	1 (Toxicity)	70	7	-	12
	2 (Toxicokinetic)	70	7	18	-
	2 (Toxicokinetic)	70	7	-	18
3 (Intermediate)	1 (Toxicity)	135	13.5	12	-
	1 (Toxicity)	160	16	-	12
	2 (Toxicokinetic)	135	13.5	18	-
	2 (Toxicokinetic)	160	16	-	18
4 (High)	1 (Toxicity)	200	20	12	-
	1 (Toxicity)	250	25	-	12
	2 (Toxicokinetic)	200	20	18	-
	2 (Toxicokinetic)	250	25	-	18

a. Group 1 administered vehicle control only (corn oil).
b. Animals dosed at a volume of 10 mL/kg.

On Day 1 of dosing in the 13-week study all mice were dosed by oral gavage at the doses outlined above. No adverse effects were observed at the low and intermediate dose levels. At the high dose levels, approximately 22 hours after the first dose, 1 male and 2 females were found dead. Additionally, 1 female displayed clinical signs of ataxia, reduced activity, hunched posture, and tremors leading to the animal being euthanized. Eight days after the first dose, 1 female was euthanized due to immobility of the hind limbs. Dosing was suspended at the high dose level after the events in the first 4 animals. These events occurred at the end of the dosing interval therefore are not considered to correlate with C_{max} of NST-6179. However, continued dosing in the high dose level cohort was suspended and new dosing levels were evaluated the study.

Macroscopic examination of major organs and tissues in decedents did not reveal any changes that could be related to drug treatment or mis-dosing via gavage. A full histopathology report of decedents is pending but TK analysis indicates drug exposure at the well-tolerated intermediate dose levels is greater than the current stopping rules. At the high dose level, drug exposure is substantially above the current stopping rule area under the concentration-time curve (AUC) of 358,000 ng.h/mL from the 28-day monkey toxicity study. The Day 1 TK parameters in the mouse are presented in Table 2.

Table 2: Summary of the NST-6179 Toxicokinetic Parameters in the Mouse on Day 1 in the 13-week Oral (Gavage) Administration Toxicity Study (Labcorp Study Number 8423764)

Group	Dose (mg/kg)	Sex	AUC_{0-24} (ng.h/mL)
Low	70	Male	229,000
	70	Female	317,000
Intermediate	135	Male	400,000
	160	Female	587,000
High	200	Male	503,000
	250	Female	988,000

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose.

Based on these data, the dose levels were adjusted to account for the necessitated decrease in high dose group while maintaining an adequate dose separation between the 3 dosing cohorts. The adjusted dose levels for both males and females are 70 mg/kg/day, 120 mg/kg/day, and 170 mg/kg/day, with the adjustments made in the ongoing low and intermediate dose cohorts and a new cohort initiated at the high dose level.

The observed adverse events (AEs) in the mouse have been considered and, as TK data indicate AEs on Day 1 correlate with exposure (AUC) substantially above the current stopping rule, are not considered to be clinically relevant.

1.5. Summary of Non-clinical Pharmacokinetics

1.5.1. Rat

Toxicokinetic parameters in rats following a single dose and repeated dosing for 28 days are presented in Table 3.

Exposure to NST-6179, as assessed by C_{max} and AUC_{0-24}, increased with increasing dose level and decreased between Day 1 and Week 4, which was consistent with hepatocellular hypertrophy and suggestive of metabolic induction.

Table 3: Summary of the NST-6179 Toxicokinetic Parameters in Rat

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	t_{max} (hours)	$t_{½}$ (hours)
1	100	Male	292,000	44,400	0.500	6.68
		Female	288,000	56,200	0.500	5.00
		Male and female	290,000	50,300	0.500	5.73
	245	Male	726,000	124,000	0.500	6.41
		Female	849,000	143,000	0.500	4.32
		Male and female	788,000	134,000	0.500	5.23
	600[a]	Male	2,000,000	274,000	0.500	NR
		Female	1,950,000	298,000	0.500	NR
		Male and female	1,990,000	286,000	0.500	NR
28 (Week 4)	100	Male	221,000	20,100	0.500	2.21
		Female	189,000	17,400	4.00	3.07
		Male and female	205,000	16,900	4.00	2.62
	245	Male	670,000	86,100	0.500	2.38
		Female	756,000	82,200	1.00	3.18
		Male and female	713,000	66,800	0.500	2.78
	450[a]	Male	1,160,000	95,400	0.500	2.32
		Female	1,030,000	66,500	9.00	NR
		Male and female	1,090,000	69,400	0.500	3.07

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose; C_{max} = maximum observed plasma concentration; NR = not reported; $t_{½}$ = apparent plasma terminal elimination half-life; TK = toxicokinetic(s); t_{max} = time of the maximum observed plasma concentration.
NOTE: combined male and female parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for TK analysis. These parameters are not an average of the values calculated for males and females separately.
a. Animals in the high dose group were dosed 600 mg/kg/day on Days 1 and 2 of the dosing phase, underwent a 2-day washout period and recommenced dosing at 450 mg/kg/day from Day 5 of the dosing phase.

1.5.2. Monkey

Toxicokinetic parameters in cynomolgus monkeys following a single dose and repeated dosing for 28 days are presented in Table 4.

Exposure to NST-6179, as assessed by C_{max} and AUC_{0-24} values, increased with the increasing dose level and in general decreased between Day 1 and Week 4 of the dosing phases suggesting some metabolic induction, consistent with the increased liver weight and microscopic findings of minimal hepatocyte hypertrophy.

Table 4: Summary of the NST-6179 Toxicokinetic Parameters in Monkey

Day	Dose (mg/kg/day)	Sex	AUC_{0-24} (ng.h/mL)	C_{max} (ng/mL)	t_{max} (hours)	$t_{½}$ (hours)
1	60	Male	116,000	57,600	0.500	2.37
		Female	121,000	93,300	0.500	2.28
		Male and female	119,000	75,400	0.500	2.33
	175	Male	403,000	111,000	0.500	4.95
		Female	518,000	155,000	1.00	2.37
		Male and female	461,000	133,000	1.00	3.66
	500 (Phase 1)[a]	Male	2,080,000	323,000	2.00	3.34
		Female	2,300,000	320,000	2.00	3.67
		Male and female	2,190,000	322,000	2.00	3.45
	350 (Phase 2)[a]	Male	990,000	228,000	1.00	5.23
		Female	976,000	165,000	1.00	3.18
		Male and female	983,000	196,000	1.00	4.54
28 (Week 4)	60	Male	92,600	52,000	1.00	4.50
		Female	98,800	72,400	0.500	3.13
		Male and female	95,700	62,200	0.500	3.96
	175	Male	133,000	40,900	0.500	3.31
		Female	358,000	147,000	1.00	4.38
		Male and female	245,000	94,000	1.00	3.85
	350 (Phase 2)[a]	Male	638,000	145,000	1.50	4.72
		Female	440,000	59,100	3.50	3.95
		Male and female	539,000	102,000	2.00	4.28

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose; C_{max} = maximum observed plasma concentration; QD = once daily; $t_{½}$ = apparent plasma terminal elimination half-life; TK = toxicokinetic(s); t_{max} = time of the maximum observed plasma concentration.
NOTE: combined male and female parameters were calculated by combining concentration data for all animals (male and female) at each dose level on each interval and using these data as a separate composite profile for TK analysis. These parameters are not an average of the values calculated for males and females separately.
a. Animals administered 500 mg/kg/day from Days 1 through 6 of the dosing phase (Phase 1) underwent a 30-day washout period and recommenced dosing with a dose level of 350 mg/kg/day (Phase 2) for 28 days (500/350 mg/kg/day).

1.5.3. Mouse

NST-6179 was administered as a single dose of 70 mg/kg to mice (the approximate effective dose), and the mean pharmacokinetic (PK) parameters are summarised in Table 5.

Table 5: Pharmacokinetic Parameters of NST-6179 in the Mouse following a Single Dose of 70 mg/kg

Sex	Dose Level (mg/kg)	C_{max} (ng/mL)	t_{max} (hours)	AUC_{0-24} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	$t_{½}$ (hours)
Male	70	36,900	0.500	188,000	227,000	7.71
Female	70	43,800	0.500	190,000	NR	11.2

Abbreviations: $AUC_{0-\infty}$ = area under the concentration-time curve from time zero to infinity; AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose; C_{max} = maximum observed plasma concentration; NR = not reported; $t_{½}$ = apparent plasma terminal elimination half-life; t_{max} = time of the maximum observed plasma concentration.

This single dose PK study in the mouse at the approximate effective dose of 70 mg/kg indicated high plasma maximum concentrations and systemic exposure (as assessed by C_{max} and AUC_{0-24}). NST-6179 was absorbed with the median time of the maximum observed concentration (t_{max}) occurring at 0.5 hours postdose in both male and female animals. Mean values for apparent terminal elimination half-life ($t_{½}$) were 7.71 hours and 11.2 hours for male and female animals, respectively. Sex ratios (male/female) for C_{max} and AUC_{0-24} were 0.842 and 0.989, respectively, indicating no notable difference in PK between male and female animals. Toxicokinetics in the 28-day rat and monkey studies indicated NST-6179 maximum plasma concentrations and systemic exposure were notably higher than the mouse.

1.6. Summary of Clinical Experience

1.6.1. Phase 1 Single Ascending Dose and Multiple Ascending Dose Study in Healthy Subjects (Study NST-6179-01, ongoing)

The study remains blinded to treatment assignment for individual subjects. However, clinical PK and safety are available from the completed cohorts and are presented here to support the continued dose escalation in this ongoing study.

1.6.1.1. Clinical Pharmacokinetics

The PK data of NST-6179 were evaluated for all completed dosing cohorts and reviewed as part of the dose escalation meetings for both the single and repeated dose cohorts. Single doses of 50 mg, 200 mg, 400 mg, 600 mg, and 1000 mg were tested under fasted conditions. Repeat dosing performed under fasting conditions at the 200 mg, 400 mg, and 1000 mg dosing levels QD for 14 days. Standard PK parameters, accumulation ratio and dose proportionality were evaluated as appropriate. The PK parameters are presented for the completed single and repeated dose cohorts in Table 6 and Table 7, respectively.

Table 6: Geometric Mean (%CV) PK Parameters of NST-6179 Following Single Oral Administration of NST-6179 Under Fasting Conditions (Part A)

Parameter (Units)	NST-6179				
	50 mg	200 mg	400 mg	600 mg	1000 mg
Cohort	A1 N=6	A2 N=6	A3 N=6	A4 N=6	A5 N=6
$AUC_{0\text{-tlast}}$ (ng*h/mL)	2490 (22.3)	10400 (29.0)	27200 (20.5)	39900 (23.0)	71400 (17.2)
$AUC_{0\text{-}\infty}$ (ng*h/mL)	2680 (16.4)	10500 (28.6)	27200 (20.5)	40000 (23.0)	71500 (17.2)
$AUC_{0\text{-}24}$ (ng*h/mL)	2680 (16.4)	10500 (28.6	27200 (20.5)	40000 (23.0)	71400 (17.2)
C_{max} (ng/mL)	1100 (54.7)	4880 (49.9)	13200 (29.2)	18300 (33.7)	26900 (33.7)
t_{max}[a] (h)	1.50 (1.00–4.00)	1.75 (1.00-4.00)	2.00 (1.50-3.00)	1.50 (0.500-2.00)	2.00 (1.00-3.00)
$t_{½}$ (h)	0.844 (8.6)	1.05 (59.2)	1.38 (40.4)	1.71 (50.2)	2.91 (76.2)

[a] Median (min-max) presented.
N = number of subjects.

Table 7: Geometric Mean (%CV) PK Parameters of NST-6179 Following Repeated Oral Administration of NST-6179 Under Fasting Conditions (Part B)

Parameter (Units)	NST-6179					
	200 mg QD		400 mg QD		1000 mg QD	
Cohort	B1		B2		B3	
	Day 1 N=8	Day 14 N=8	Day 1 N=8	Day 14 N=8	Day 1 N=8	Day 14 N=8
$AUC_{0\text{-}\tau}$ [a] (ng*h/mL)	11300 (41.5)	12900 (31.6)	21900 (36.6)	24700 (26.7)	71100 (16.0)	85700 (19.8)
$AUC_{0\text{-}\infty}$ (ng*h/mL)	11300 (41.4)	NA	21900 (36.6)	NA	71100 (16.0)	NA
C_{max} (ng/mL)	5690 (51.0)	6920 (28.0)	10500 (60.4)	10000 (34.9)	29100 (25.3)	31800 (39.9)
t_{max}[b] (h)	1.25 (1.00-3.00)	1.25 (1.00-2.00)	2.00 (1.00-3.00)	1.50 (1.00-3.00)	2.00 (1.00-4.00)	2.00 (1.00-2.00)
$t_{½}$ (h)	1.04 (37.2)	1.01 (16.6)	1.19 (31.5)	1.64 (113.0)	2.33 (56.5)	3.22 (20.0)
C_{min} (ng/mL)	NA	NC	NC	NC	NA	8.10 (28.9)
$RA_{AUC0\text{-}\tau}$	NA	1.14 (15.5)	NA	1.13 (18.7)	NA	1.21 (17.6)
RA_{Cmax}	NA	1.22 (32.3)	NA	0.953 (46.7)	NA	1.09 (39.9)

Note: C_{min} values are NC because the minimum concentration in all profiles was 0, and geometric mean was not calculated.
[a] Refers to a $AUC_{0\text{-}24}$ for Day 1.
[b] Median (min-max) presented.
N = number of subjects; NA = not applicable; NC = not calculated; QD = once daily; RA = accumulation ratio.

To date, none of the subjects receiving single or repeat administration of NST-6179 have exceeded the PK stopping criteria. Following single administration of 1000 mg NST-6179 (Cohort A5), the highest individual systemic NST-6179 exposure represented 73.8% and 25.2% of the stopping criteria for C_{max} and $AUC_{0\text{-}24}$, respectively. Following repeat

administration of 1000 mg NST-6179 QD for 14 days (Cohort B3), the highest individual systemic NST-6179 exposure represented 88.2% and 34.1% of the PK stopping criteria, based on C_{max} and AUC_{0-24}, respectively.

Overall, PK across the dose range of 50 to 1000 mg was generally consistent, with median t_{max} between 1 to 2 hours postdose, estimates for $t_{1/2}$ in the range of 1 to 3 hours, and no evidence of accumulation following QD administration. From 50 to 1000 mg, AUC and C_{max} increased in a manner that was generally proportional (or slightly more than proportional) to the increase in dose; as dose increased by 20-fold, geometric mean C_{max} and $AUC_{0-\infty}$ increased by 24- to 26-fold, respectively.

1.6.1.2. Clinical Safety

A total of 70 subjects have been exposed to either NST-6179 or matched placebo in single doses up to 1000 mg and as multiple doses up to 1000 mg QD for 14 days. The study is ongoing and remains blinded, but analysis of the adverse events (AEs) has been performed as part of dose escalation reviews. A total of 89 AEs were reported in 31 subjects during the study period, the majority of which were mild (77 of the 89 AEs) and considered not related to NST-6179 treatment (54 of the 89 AEs). There were no severe or serious AEs reported during the study period. The most common AE (greater than 2 subjects) considered to be related to NST-6179 treatment was headache; 15 AEs reported by 7 subjects. There were no clinically significant changes or abnormalities in laboratory parameters, vital signs, ECG parameters, or physical examinations recorded at for any of the doses of NST-6179 administered. In summary, NST-6179 appears to be safe and well tolerated at single doses up to 1000 mg and repeated doses of 1000 mg QD for up to 14 days.

1.7. Study Rationale

This is the first time NST-6179 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when NST-6179 is administered orally as single and multiple doses to healthy subjects. This information will help establish the doses and dosing regimen suitable for future studies in patients.

1.8. Benefit-risk Assessment

Subjects in the current study will not receive any health benefit (beyond that of an assessment of their medical status) from participating in the study. The risks of participation are primarily those associated with adverse reactions to the study treatment, although there may also be some discomfort from collection of blood samples and other study procedures.

The events observed in the toxicology studies to date are also considered in determining the overall benefit-risk of the Phase 1 study. All events occurred in these specific studies at systemic exposure exceeding the current established stopping rules. Based on the observed drug exposure, a significant safety margin exists for both the relevant species, as well as the mice in the carcinogenicity dose ranging study, at the proposed initial human dose of 50 mg. Therefore, the risk-benefit of conducting this first-in-human study remains unchanged and proposed stopping rules remain supported by data from the mouse, rat, and monkey.

More information about the known and expected benefits, risks, and reasonably anticipated AEs associated with NST-6179 may be found in the Investigator's Brochure (IB).[1]

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objectives of the study are:

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

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

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.
- to evaluate the effect of pharmacogenomics (PGx) on the PK of NST-6179 in healthy male and female subjects.

2.2. Endpoints

2.2.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

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

2.2.2. 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 zero to infinity ($AUC_{0-\infty}$)
- area under the concentration-time curve from time zero to infinity normalised by dose ($AUC_{0-\infty}$/dose)

- area under the concentration-time curve from time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$)
- area under the concentration-time curve from time zero to the time of the last quantifiable concentration normalised by dose ($AUC_{0\text{-}tlast}$/dose)
- C_{max}
- C_{max} normalised by dose (C_{max}/dose)
- t_{max}
- $t_{½}$
- apparent total clearance (CL/F)
- apparent volume of distribution (V_z/F)
- amount of drug excreted (A_e)
- percentage of dose excreted (f_e)
- renal clearance (CL_R).

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

- area under the concentration-time curve over a dosing interval ($AUC_{0\text{-}\tau}$)
- AUC from time zero to 24 hours postdose ($AUC_{0\text{-}24}$, derived as AUC0 τ*2 for a BID dosing regimen at steady state)
- area under the concentration-time curve over a dosing interval normalised by dose ($AUC_{0\text{-}\tau}$/dose)
- $AUC_{0\text{-}\infty}$ (Day 1 only)
- $AUC_{0\text{-}\infty}$/dose (Day 1 only)
- C_{max}
- C_{max}/dose
- t_{max}
- $t_{½}$
- CL/F
- V_z/F
- minimum observed concentration (C_{min})
- observed accumulation ratio based on $AUC_{0\text{-}\tau}$ ($RA_{AUC0\text{-}\tau}$)
- observed accumulation ratio based on C_{max} (RA_{Cmax}).

Other PK parameters may also be added.

2.2.3. Exploratory Endpoints

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

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

Optional PGx samples will be collected from subjects that consent, for the determination of common gene variants (ie, cytochrome P450 2C9), that may have an effect on the PK of NST-6179.

3. INVESTIGATIONAL PLAN

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.

3.1. Overall Study Design and Plan

3.1.1. 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.

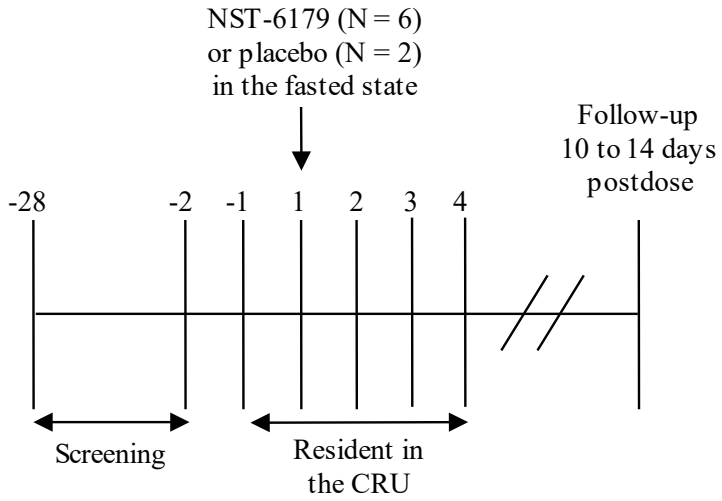
Additional Groups (Groups A7 to A9)
If it is decided to enrol additional groups (Section 3.3) fasting requirements, meal compositions, and timing of doses will be determined following review of the available PK data.

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.

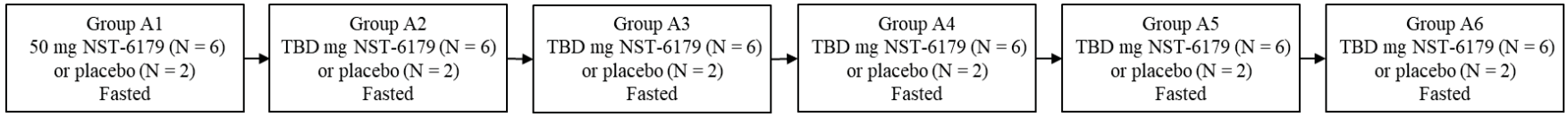
An overview of the study design is shown in Figure 1 and the planned dose levels are presented in Figure 2. A Schedule of Assessments is presented in Appendix 6.

Figure 1: Study Schematic (Part A) for Groups A1 to A6



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

Figure 2: Planned Dose Levels for 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.

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

3.1.2. 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.

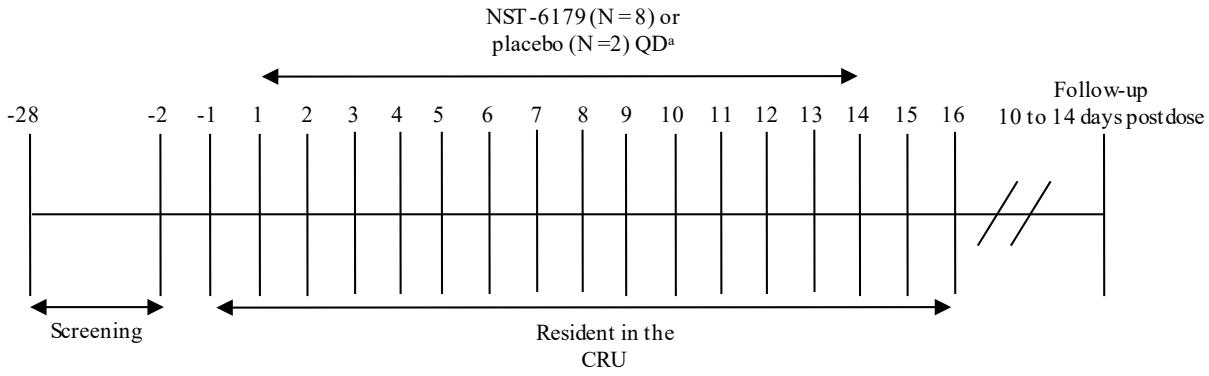
Based on the ongoing review of the safety, tolerability, and PK results, additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 per period and will not extend beyond 28 days after each final dosing occasion.

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 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 (Section 3.4). 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 (Section 3.4). There will be a minimum of 14 days between dose escalations for each group in Part B. The dose selected on each dosing occasion in Part B will not exceed a single dose shown to be safe and well tolerated in Part A. Sentinel dosing will be implemented for any groups in Part B for which the total daily dose exceeds the highest single dose evaluated in Part A (eg, 1000 mg BID for which the total daily dose of 2000 mg has not been administered as a single dose in Part A), whereby an initial cohort of 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining cohort of 8 subjects (7 active and 1 placebo) will be dosed after at least 72 hours.; Section 3.4).

Up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B (Section 3.3).

An overview of the study design is shown in Figure 3, and the planned dose levels are presented in Figure 4. A Schedule of Assessments is presented in Appendix 6.

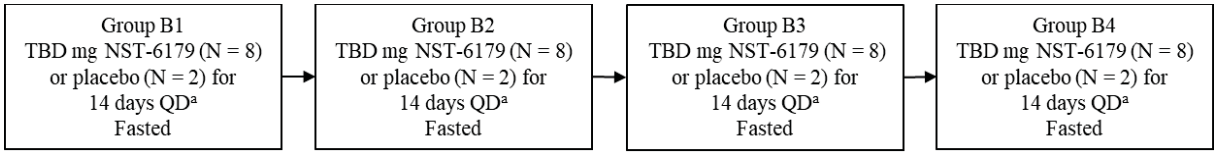
Figure 3: Study Schematic for Part B



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. 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.

Figure 4: Planned Dose Levels for 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. 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.

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

A Schedule of Assessments is presented in Appendix 6.

3.2. Study Start and End of Study Definitions

The start of the study is defined as the date the first subject signs an informed consent form (ICF). The point of enrolment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

3.3. Additional Groups

Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7] for Part A or for Part B where the dose of NST 6179 will not exceed a single

dose shown to be safe and well tolerated in Part A) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A, and up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B. There will be no further dose escalation in these additional groups if dose escalation stopping criteria have been met and a dose level cannot be repeated if it previously met a stopping criterion. The requirement for additional groups will be agreed with the sponsor and documented in the trial master file (TMF).

3.4. Discussion of Study Design, Including the Choice of Control Groups

For Part A and Part B of the study, a sequential-group, ascending-dose design has been chosen for safety reasons as NST-6179 is in the early stages of clinical development, with Part A of the study being the first time it will be administered to humans. Oral doses have been chosen for both parts of the study, as this is the intended clinical route of administration.

For safety reasons, sentinel dosing will be used in Part A, such that 2 subjects (1 NST-6179 and 1 placebo) will be dosed 24 hours before the remaining 6 subjects. Sentinel dosing is not considered necessary in Part B when the total daily exposure is not be predicted to exceed an exposure previously shown to be safe and well tolerated in Part A. Sentinel dosing will be implemented for any groups in Part B for which the total daily dose (based on multiple doses per day) exceeds the highest single dose evaluated in Part A (eg, 1000 mg BID for which the total daily dose of 2000 mg has not been administered as a single dose in Part A).

It is the intent of Part B to dose subjects such that steady-state plasma levels of NST-6179 are achieved and maintained for several days. Based on the available non-clinical data, it is expected that this will be achieved following 14 days of QD dosing; however, a full review of all the safety, tolerability, and PK data from Part A will be performed to confirm the dose regimen for Part B. If the $t_{½}$ of NST-6179 is shorter than predicted by the non-clinical data, up to 4-times-daily dosing over 7 days may be appropriate. If the $t_{½}$ of NST-6179 is longer than predicted by the non-clinical data, QD dosing may be more appropriate over 28 days (as steady state will take longer to achieve). 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.

Details of the dosing regimen and duration used for Part B of the study will be documented in the TMF.

Based upon the non-clinical data, the duration of each treatment period is considered adequate to achieve the study objectives.

Part A and Part B will be double-blind and placebo-controlled in order to avoid bias in the collection and evaluation of data during its conduct. Placebo has been chosen as the control treatment to assess whether any observed effects are treatment-related or simply reflect the study conditions.

Conducting the study in healthy subjects mitigates the potential confounding effects of the disease state and concomitant medications.

Continuous 12-lead ECG monitoring will be included in this study in order to explore potential NST-6179 effects on the QT interval, with a view of supporting a potential thorough QT study waiver.

3.4.1. Dose Interval Between Subjects

Following thorough review of all available non-clinical data (pharmacological and toxicological), dosing at each dose level in Part A will be such that 2 subjects (1 NST-6179 and 1 placebo) will be dosed 24 hours before the remaining 6 subjects. After dosing the first 2 subjects on a separate day, a minimum of a 5-minute dosing interval for the remaining 6 subjects in each dose-ascending group is considered acceptable. Continuation to dose the remaining 6 subjects will be at the investigator's discretion.

In Part B, a minimum of a 5-minute dosing interval between subjects in each group is considered acceptable.

In Part B, for any groups for which the total daily dose exceeds the highest single dose evaluated in Part A (eg, 1000 mg BID for which the total daily dose of 2000 mg has not been administered as a single dose in Part A), dosing will be such that a cohort of 2 subjects (1 NST-6179 and 1 placebo) will be dosed at least 72 hours before the remaining 8 subjects.

3.5. Selection of Doses in the Study

In the 28-day repeat dose toxicology studies, NOAELs in the rat and cynomolgus monkey were 450 mg/kg and 350 mg/kg, respectively. These correspond to human equivalent doses (HEDs) of:

Rat: 450 mg/kg × 0.16 = **72 mg/kg**
(equivalent to approximately 4320 mg in a 60-kg subject)

Monkey: 350 mg/kg × 0.32 = **112 mg/kg**
(equivalent to approximately 6720 mg in a 60-kg subject)

Where 0.16 and 0.32 are conversion factors to extrapolate the animal dose to the HED based on body surface area.[2]

The rat was the most sensitive species (ie, that with the lowest HED), and assuming a 10-fold safety margin, this equates to a maximum recommended starting dose (MRSD) of:

$$\frac{72 \text{ mg/kg}}{10} = 7.2 \textbf{ mg/kg}$$

or 7.2 mg/kg × 60 kg = **432 mg** in a 60-kg subject

Compared to the HED for the NOAEL in the rat, the most sensitive species (ie, that with the lowest HED), a starting dose of 50 mg in Part A will correspond to a dose of:

$$\frac{50 \text{ mg}}{60 \text{ kg}} = 0.83 \text{ mg/kg in a 60-kg subject}$$

and a safety margin of:

$$\frac{72 \text{ mg/kg}}{0.83 \text{ mg/kg}} = \mathbf{87\text{-fold}}$$

The approximate effective dose of NST-6179 in an efficacy model in the mouse was 70 mg/kg (Section 1.5.3), which corresponds to a HED of:

Mouse: 70 mg/kg × 0.08 = **5.6 mg/kg**
(equivalent to approximately 336 mg in a 60-kg subject)

Where 0.08 is the conversion factor to extrapolate the animal dose to the HED based on body surface area.[2] Compared to the HED for the approximate effective dose in the mouse (ie, dose shown to have pharmacological activity in the mouse model), a starting dose of 50 mg in Part A will be approximately 7-fold lower than a dose expected to have pharmacological activity:

$$\frac{5.6 \text{ mg/kg}}{0.83 \text{ mg/kg}} = \mathbf{6.7\text{-fold}}$$

Evaluation of protein binding indicates the unbound drug fraction is 4% in mouse compared to 1.78% in human. The proposed starting dose based on unbound drug is therefore approximately 15 times lower than the effective dose. Although there is no empirical data that defines a pharmacologically inactive dose in the mouse it is considered unlikely that a human starting dose of 50 mg would elicit significant pharmacological activity, if any. Furthermore metabolism *in vitro* is notably lower in mouse compared to human. Exposure to parent drug in human is likely to be proportionately less than mouse based on equivalent doses.

The proposed investigational medicinal product (IMP) dose levels are shown in Table 8. However, based on the ongoing review of the available safety, tolerability, and PK data, as applicable, doses may be adjusted.

Table 8: Investigational Medicinal Product Dose Levels for Parts A and B

Study Part	Group	Subject Numbers	Treatment Period 1
Part A	A1	0101 – 0108	50 mg or placebo
	A2	0109 – 0116	200 mg or placebo
	A3	0117 – 0124	400 mg or placebo
	A4	0125 – 0132	600 mg or placebo
	A5	0133 – 0140	1000 mg or placebo
	A6	0141 – 0148	TBD mg or placebo
	A7[a]	0149 – 0156	TBD
	A8[a]	0157 – 0164	TBD
	A9[a]	0165 – 0172	TBD
Part B	B1	0201 – 0210	200 mg or placebo QD
	B2	0211 – 0220	400 mg or placebo QD
	B3	0221 – 0230	1000 mg or placebo QD
	B4	0231 – 0240	TBD mg or placebo BID
	B5[b]	0241 – 0250	TBD
	B6[b]	0251 – 0260	TBD
	B7[b]	0261 – 0270	TBD

Abbreviations: BID = twice daily; NA = not applicable; PK = pharmacokinetic(s); QD = once daily; TBD =to be determined.
a. Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A.
b. Following review of the safety, tolerability, and PK data, additional dose groups (where the dose of NST 6179 will not exceed a single dose shown to be safe and well tolerated in Part A) may be added to the study. Up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B.

For Part B of the study, dose levels, dosing frequency, and dosing duration will be decided, in consultation with the sponsor, on the basis of data from Part A of the study or earlier groups in Part B. The dose of NST-6179 per dosing occasion in Part B will not exceed a single dose shown to be safe and well tolerated in Part A.

Dose levels should not increase by more than 5-fold for predicted non-pharmacologically active dose levels and by 3-fold for doses above 300 mg (ie, the predicted pharmacologically active dose level).

Dose levels may be increased, providing systemic exposure does not exceed that stated in the dose escalation stopping criteria (Section 3.7).

Details of all doses administered in Part A and Part B of the study will be documented in the TMF.

3.6. Dose Escalation

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. Doses may be reduced and may be lower than the starting dose. There will be a minimum of 7 days between dose escalations in Part A and a minimum of 14 days between dose escalations in Part B to allow sufficient time for an adequate safety review for each respective part.

Dose escalation in both Part A and Part B will only occur if data from a minimum of 6 subjects have been reviewed from the previous dose group, such that data from a minimum of 4 subjects who have received NST-6179 will be used to make the dose escalation decision.

The justification for this is as follows:

- The study treatment is of a known pharmacological class for which the on-target effects in humans are well characterised. Based upon non-clinical data, no clinically important off-target effects are expected within the proposed dose range.
- A minimum of 4 subjects receiving the active drug is considered sufficient to characterise the safety profile and PK response to NST-6179.

Between each dose escalation, the investigator will review all available data in a blinded manner to ensure it is safe to proceed with the planned dose escalation. An interim safety report, summarising results from all available safety assessments, will be sent to the sponsor prior to the start of each successive group/treatment period. Any clinically significant results will be discussed with the sponsor before dose escalation continues. Interim PK data will also be reviewed in terms of dose escalation and to confirm that the study design remains appropriate. In the event of a disagreement between the sponsor and investigator on the dose escalation decision, the decision of the investigator will be upheld.

3.7. Dose Escalation Stopping Criteria

The study will be halted if 1 or more subject experience a serious adverse event (SAE) that is considered to be related to IMP or 2 or more subjects in the same group experience severe AEs that are considered to be related to IMP. If, following an internal safety review, the sponsor deems it appropriate to restart the study, this can be done following approval of a substantial protocol amendment. Dosing of subjects, including any ongoing multiple-dose groups, will be stopped immediately if any of the dose escalation stopping criteria are met.

In Part A and Part B, following consultation with the sponsor, dose escalation will stop if:

- Clinically relevant signs or symptoms of similar nature occur in 2 or more subjects in a group that, in the opinion of the investigator, warrant stopping of dose escalation.
- There is evidence of clinically significant increases in liver function tests defined as 3 times the upper limit of normal (ULN) for aspartate aminotransferase (AST), ALT, ALP, or gamma-glutamyl transferase (GGT) or 2 times the ULN for total bilirubin in 2 or more subjects in a group (confirmed with repeat testing).
- Moderate nausea or vomiting that prevents subjects from eating a meal on 3 or more occasions on 2 consecutive days in 2 or more subjects in a group.
- The systemic exposure for any individual subject is predicted to exceed an $AUC_{0\text{-}24}$ of 358,000 ng.hr/mL (based on the female cynomolgus monkey at the intermediate dose level of 175 mg/kg/day at Day 28) in the 28-day oral toxicity study and/or a C_{max} of 59,100 ng/mL (based on the female cynomolgus monkey kinetics at the NOAEL [350mg/kg/day at Day 28]).

4. SELECTION OF STUDY POPULATION

4.1. Inclusion Criteria

4.1.1. Part A and Part B

Subjects must satisfy all of the following criteria at the screening visit unless otherwise stated:

1. Males or females, of any race, between 18 and 65 years of age, inclusive.
2. Body mass index between 18.0 and 32.0 kg/m^2, inclusive.
3. In good health, determined by no clinically significant findings from medical history, physical examination, 12-lead ECG, vital signs measurements, and clinical laboratory evaluations (congenital non-haemolytic hyperbilirubinemia [eg, suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable) at screening and/or check-in as assessed by the investigator (or designee).
4. Females will not be pregnant or lactating, and females of childbearing potential and males will agree to use contraception as detailed in Appendix 4.
5. Able to comprehend and willing to sign an ICF and to abide by the study restrictions.

4.2. Exclusion Criteria

4.2.1. Part A and Part B

Subjects will be excluded from the study if they satisfy any of the following criteria at the screening visit unless otherwise stated:

Medical Conditions

1. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, haematological, pulmonary, cardiovascular, gastrointestinal, neurological, respiratory, endocrine, or psychiatric disorder, as determined by the investigator (or designee).
2. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the investigator (or designee).
3. History of stomach or intestinal surgery or resection that would potentially alter absorption and/or excretion of orally administered drugs (uncomplicated appendectomy and hernia repair will be allowed).
4. Any of the following:
    a. QT interval corrected for heart rate using Fridericia's method (QTcF) > 450 ms confirmed by repeat measurement.
    b. QRS duration > 110 ms confirmed by repeat measurement.

c. PR interval > 220 ms confirmed by repeat measurement.

d. findings which would make QTc measurements difficult or QTc data uninterpretable.

e. history of additional risk factors for torsades de pointes (eg, heart failure, hypokalaemia, family history of long QT syndrome).

5. Confirmed (eg, 2 consecutive measurements) systolic blood pressure > 140 mmHg or < 80 mmHg, diastolic blood pressure > 90 mmHg or < 45 mmHg, and pulse rate > 100 beats per minute (bpm) or < 40 bpm.
6. Positive hepatitis panel and/or positive human immunodeficiency virus test (Appendix 2).

Prior/Concomitant Therapy

7. Administration of a Coronavirus Disease 2019 (COVID-19) vaccine in the past 28 days prior to dosing.
8. Use or intend to use any medications/products known to alter drug absorption, metabolism, or elimination processes, including St. John's wort, within 30 days prior to dosing, unless deemed acceptable by the investigator (or designee).
9. Use or intend to use any prescription medications/products other than hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives within 14 days prior to dosing, unless deemed acceptable by the investigator (or designee).
10. Use or intend to use slow-release medications/products considered to still be active within 14 days prior to check-in, unless deemed acceptable by the investigator (or designee).
11. Use or intend to use any non-prescription medications/products including vitamins, minerals, and phytotherapeutic/herbal/plant-derived preparations within 7 days prior to check-in, unless deemed acceptable by the investigator (or designee).

Prior/Concurrent Clinical Study Experience

12. Participation in a clinical study involving administration of an investigational drug (new chemical entity) in the past 90 days prior to dosing.
13. Have previously completed or withdrawn from this study.

Diet and Lifestyle

14. Alcohol consumption of > 14 units per week. One unit of alcohol equals ½ pint (285 mL) of beer or lager, 1 glass (125 mL) of wine, or 1/6 gill (25 mL) of spirits.
15. Positive alcohol breath test result or positive urine drug screen (confirmed by repeat) at screening or check-in.
16. History of alcoholism or drug/chemical abuse within 2 years prior to check-in.
17. Use of tobacco- or nicotine-containing products within 3 months prior to check-in, or positive cotinine at screening or check-in.

18. Ingestion of poppy seed-, Seville orange-, or grapefruit-containing foods or beverages within 7 days prior to check-in.

Other Exclusions

19. Receipt of blood products within 2 months prior to check-in.
20. Donation of blood from 3 months prior to screening, plasma from 2 weeks prior to screening, or platelets from 6 weeks prior to screening.
21. Poor peripheral venous access.
22. Subjects who, in the opinion of the investigator (or designee), should not participate in this study.
23. Mental incapacity or language barriers which preclude adequate understanding or cooperation, or unwillingness to comply with trial requirements.
24. An employee of the sponsor or investigator or otherwise dependent on them.

4.3. Generic Screening

Subjects may previously have been screened on a generic basis to determine their eligibility for inclusion in Phase 1 clinical studies conducted at the study site. If generic screening was performed within the specified study screening window, selected study-specific procedures will be repeated either at an additional screening visit or on admission to the study site on Day -1.

4.4. Subject Number and Identification

Subjects will have a unique identification number used at screening. Subjects will be assigned a subject number at the time of their randomisation. Assignment of subject numbers will be in ascending order and no numbers will be omitted (eg, in Part A, Subjects 0101, 0102, etc; in Part B, Subjects 0201, 0202, etc). Replacement subjects (Section 4.5) will be assigned a subject number corresponding to the number of the subject he/she is replacing plus 1000 (eg, Subject 1101 replaces Subject 0101).

Subjects will be identified by screening identification number or subject number only on all study documentation. A list identifying the subjects by subject number will be kept in the Site Master File. This list will only contain subject number, sex, age, year of birth, and Trial One ID of subjects (ie, Screening Number). No identifiable confidential information can be filed.

4.5. Subject Withdrawal and Replacement

A subject is free to withdraw from the study at any time. In addition, a subject will be withdrawn if any of the following criteria are met:

- change in compliance with any inclusion/exclusion criterion that is clinically relevant and affects subject safety as determined by the investigator (or designee)
- non-compliance with the study restrictions that might affect subject safety or study assessments/objectives, as considered applicable by the investigator (or designee)

- any clinically relevant sign or symptom that, in the opinion of the investigator (or designee), warrants subject withdrawal.

If a subject is withdrawn from dosing, the sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the subject's electronic case report form (eCRF). If a subject is withdrawn from the study, efforts will be made to perform all follow-up assessments, if possible (Appendix 6). Other procedures may be performed at the investigator's (or designee's) and/or sponsor's discretion. If the subject is in-house, these procedures should be performed before the subject is discharged from the clinic. The investigator (or designee) may also request that the subject return for an additional follow-up visit. All withdrawn subjects will be followed until resolution of all their AEs or until the unresolved AEs are judged by the investigator (or designee) to have stabilised.

Subjects who are withdrawn for reasons not related to study treatment may be replaced following discussion between the investigator and the sponsor. Subjects withdrawn as a result of AEs thought to be related to the study treatment will generally not be replaced.

4.6. Study Termination

The study may be discontinued at the discretion of the investigator (or designee), sponsor, or sponsor's medical monitor if any of the following criteria are met:

- AEs unknown to date (ie, not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)
- increased frequency, severity, and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at check-in as baseline signs and symptoms)
- medical or ethical reasons affecting the continued performance of the study
- difficulties in the recruitment of subjects
- cancellation of drug development.

5. STUDY TREATMENTS

5.1. Description, Storage, Packaging, and Labelling

The IMPs (10 mg and 100 mg NST-6179 capsule and placebo) will be supplied by the sponsor (or designee), along with the batch/lot numbers and certificates of analysis. A certificate of release authorised by a Qualified Person from a listed country or United Kingdom will also be issued for the IMP.

All IMPs will be stored at the study site at ambient temperature (< 30°C) in a location that is locked with restricted access.

NST-6179 will be supplied as a solid in hard gelatine capsules, 10 mg or 100 mg. Placebo will be supplied in matching hard gelatine capsules. The 10-mg formulation contains NST-6179 drug substance formulated in microcrystalline cellulose and magnesium stearate.

The 100-mg formulation contains NST-6179 only. The placebo contains microcrystalline cellulose and magnesium stearate.

The bulk drug container and unit dose containers will be labelled in accordance with national laws and regulations. The IMPs will be transferred from bulk supplies into the subject's dose container by qualified clinical staff.

5.2. Study Treatment Administration

Full details on dietary restrictions for dose administration are described in Section 6.2.

Each dose of NST-6179 and placebo will be administered orally with approximately 240 mL of room temperature water. In Part A and on the days with intensive PK assessments in Part B, all doses will be administered after an overnight fast of at least 10 hours. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing.

For any groups in Part B where a BID dosing regimen will be implemented doses will be administered in the morning (AM) and evening (PM) separated by 12 ± 0.5 hours (except on Day 14, when only the AM dose will be administered). Dose administration will occur in the fasted state; subjects will fast for at least 10 hours prior to and for 4.5 hours after the morning (AM) dose on Day 1 and the final day of dosing. For the Day 1 PM dose and all other dosing occasions subjects will fast for at least 2 hours prior to dosing and 2 hours after dosing.

Subjects will be administered the IMP in numerical order while standing and will not be permitted to lie supine for 2 hours after dosing, except as necessitated by the occurrence of an AE(s) and/or study procedures.

5.3. Randomisation

The randomisation code will be produced by the statistics department at Labcorp Drug Development using a computer-generated pseudo-random permutation procedure.

In Part A, 6 subjects per group will be randomly assigned to receive NST-6179 and 2 subjects per group will be randomly assigned to receive placebo. For all groups in Part A, sentinel dosing will occur whereby 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining 6 subjects (5 active and 1 placebo) will be dosed after 24 hours.

In Part B, 8 subjects per group will be randomly assigned to receive NST-6179 and 2 subjects per group will be randomly assigned to receive placebo. If sentinel dosing will be implemented for any groups in Part B, 2 subjects (1 active and 1 placebo) will be randomised and dosed on Day 1, providing no safety concerns arise, the remaining cohort of 8 subjects (7 active and 1 placebo) will be dosed after at least 72 hours.

Prior to the start of the study, a copy of the master randomisation code will be supplied to the study site pharmacy staff (in sealed envelopes) and the biopharmaceutical analyst(s) at the bioanalytical laboratory.

5.4. Blinding

Both Part A and Part B of the study will be conducted in a double-blind manner. Treatments will be administered in a double-blind manner, and subjects will remain blinded with regard to treatment throughout the study.

The following controls will be employed to maintain the double-blind status of the study:

- The placebo capsules will be identical in appearance to NST-6179.
- The investigator and other members of staff involved with the study will remain blinded to the treatment randomisation code during the assembly procedure.
- The investigator and other members of staff (with the exception of pharmacy staff) involved with the study will remain blinded to the treatment randomisation code during the conduct of the study.
- Interim bioanalytical data will be provided to Labcorp CRU in a blinded manner.

To maintain the blind, the investigator will be provided with a sealed randomisation code for each subject, containing details of their treatment. These individual sealed envelopes will be kept in a limited access area that is accessible 24 hours a day. In order to manage subject safety or to support dose escalation decisions (in the event of treatment-related SAEs or severe AEs), the decision to unblind resides solely with the investigator. Whenever possible, and providing it does not interfere with or delay any decision in the best interest of the subject, the investigator will discuss the intended code-break with the sponsor. If it becomes necessary to break the code during the study, the date, time, and reason will be recorded in the subject's source data and on the individual envelope and will be witnessed by a second person.

5.5. Treatment Compliance

The following measures will be employed to ensure treatment compliance:

- All doses will be administered under the supervision of suitably qualified study site staff.
- Immediately after dose administration, visual inspection of the mouth and hands will be performed for each subject.
- A predose and postdose inventory of IMP will be performed on the dose containers.

5.6. Drug Accountability

The investigator (or designee) will maintain an accurate record of the receipt of the study supplies received. In addition, an accurate drug disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensing. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the sponsor upon request.

For each batch of unit doses, the empty used unit dose containers will be discarded upon satisfactory completion of the compliance and accountability procedures. Any unused assembled unit doses will be retained until completion of the study.

At the completion of the study, all unused supplies will be returned to the sponsor or disposed of by the study site, per the sponsor's written instructions.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Subjects will refrain from use of any prescription or non-prescription medications/products during the study until the follow-up visit, unless the investigator (or designee) and/or sponsor have given their prior consent.

Paracetamol/acetaminophen (2 g/day for up to 3 consecutive days), hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives are acceptable concomitant medications. The administration of any other concomitant medications during the study is prohibited without prior approval of the investigator (or designee), unless its use is deemed necessary for treatment of an AE. Any medication taken by a subject during the course of the study and the reason for its use will be documented in the source data.

A risk assessment has been performed and subjects will be permitted to receive a COVID-19 vaccine after dosing, if required. The administration of a COVID-19 vaccine will be recorded as a concomitant medication.

6.2. Diet

While confined at the study site, subjects will receive a standardised diet (as per study site procedures) at scheduled times that do not conflict with other study-related activities. Subjects will be fasted (at least 8 hours) before collection of blood samples for clinical laboratory evaluations.

On the days with intensive PK assessments, meals will be identical for each group.

On Day 1 in Part A, subjects will be fasted for at least 10 hours prior to dosing until approximately 4 hours after dosing, when lunch will be provided (lunch times on Day 1 will be staggered between subjects to ensure this). With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing. Meals will be provided as appropriate at other times. Other than the fluid restrictions on dosing days, water will be freely available at all times.

In Part B, on days with intensive PK assessments (eg, Days 1 and 14), subjects will be fasted for at least 10 hours prior to morning (AM) dosing until approximately 4.5 hours after morning (AM) dosing. On days without intensive PK assessments and for the Day 1 PM dose, subjects will also fast for at least 2 hours prior to dosing and 2 hours after dosing. Meals will be provided as appropriate at other times. With the exception of water given with the

dose, subjects will not be allowed fluids from 1 hour prior to dosing until 2 hours after dosing. Other than these fluid restrictions, water will be freely available at all times.

Foods and beverages containing poppy seeds, grapefruit, or Seville oranges will not be allowed from 7 days prior to check-in until the follow-up visit.

Caffeine-containing foods and beverages will not be allowed from 36 hours before check-in until discharge.

Consumption of alcohol will not be permitted from 36 hours prior to check-in until discharge. Up to 2 units/day of alcohol are permitted from discharge until 36 hours before the follow-up visit or 36 hours before check-in in each treatment period.

6.3. Smoking

Subjects will not be permitted to use tobacco- or nicotine-containing products within 3 months prior to check-in until the follow-up visit.

6.4. Exercise

Subjects are required to refrain from strenuous exercise from 7 days before check-in until the follow-up visit and will otherwise maintain their normal level of physical activity during this time (ie, will not begin a new exercise program nor participate in any unusually strenuous physical exertion).

6.5. Blood Donation

Subjects are required to refrain from donation of blood from 3 months prior to screening, plasma from 2 weeks prior to screening, and platelets from 6 weeks prior to screening until 3 months after the follow-up visit.

7. STUDY ASSESSMENTS AND PROCEDURES

Every effort will be made to schedule and perform the procedures as closely as possible to the nominal time, giving considerations to appropriate posture conditions, practical restrictions, and the other procedures to be performed at the same timepoint.

The highest priority procedures will be performed closest to the nominal time. The order of priority for scheduling procedures around a timepoint is (in descending order of priority):

- dosing
- blood samples (for NST-6179 assay)
- ECG extractions
- urine samples (for NST-6179 assay)
- vital signs
- any other procedures.

7.1. Pharmacokinetic Assessments

7.1.1. Sample Collection and Processing for Pharmacokinetic Assessment

Blood samples (approximately 1 × 2.0 mL) will be collected by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6. Furthermore, up to 3 additional blood samples may be taken from each subject per treatment period/PK sampling day, with the maximum volume of blood withdrawn per subject not exceeding the limit detailed in Appendix 3. Any changes to the scheduled times of PK assessments will be agreed with the sponsor and documented in the TMF. Samples taken from subjects who received placebo will not be analysed.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

Urine samples will be collected over the time intervals indicated in the Schedule of Assessments in Appendix 6. Procedures for collection, processing, and shipping of urine samples will be detailed in a separate document.

7.1.2. Sample Collection and Processing for Metabolite Identification

For subjects in the highest QD dose group in Part B, blood samples (approximately 1 × 2.0 mL) for metabolite identification will be collected by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6. Samples taken from subjects who received placebo will not be analysed. Furthermore, up to 3 additional blood samples may be taken from each subject per treatment period/PK sampling day, with the maximum volume of blood withdrawn per subject not exceeding the limit detailed in Appendix 3.

Procedures for collection, processing, and shipping of blood samples for metabolite identification will be detailed in a separate document.

Urine samples will be collected over the time intervals indicated in the Schedule of Assessments in Appendix 6. Procedures for collection, processing, and shipping of urine samples will be detailed in a separate document.

7.1.3. Analytical Methodology

Plasma and urine concentrations of NST-6179 will be determined using validated analytical procedures. Specifics of the analytical methods will be provided in separate documents.

For the highest QD dose group in Part B, an exploratory analysis of NST-6179 metabolites in plasma and urine may be performed utilising the blood and urine samples collected for PK evaluation.

7.1.4. Sample Collection and Processing for Pharmacogenomics

An optional blood sample (approximately 1 × 6 mL) for PGx testing may be collected, if the subject consents, by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6.

Samples will be kept for future research and will be stored at the Sponsor designated storage facility. The manager of these samples will ensure that they are properly used throughout their usable life and will destroy the samples at the end of the scheduled storage period, no longer than 25 years after the end of the study or the maximum period allowed by applicable law.

Further details of sample collection, processing, and storage will be provided in a separate document.

7.2. Safety and Tolerability Assessments

7.2.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting SAEs are detailed in Appendix 1.

The condition of each subject will be monitored from the time of signing the ICF to final discharge from the study. Subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as “How have you been feeling since you were last asked?”, at least once each day while resident at the study site and at each study visit. Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the study.

Any AEs and remedial action required will be recorded in the subject’s source data. The nature, time of onset, duration, and severity will be documented, together with an investigator’s (or designee’s) opinion of the relationship to study treatment.

Adverse events recorded during the course of the study will be followed up, where possible, until resolution or until the unresolved AEs are judged by the investigator (or designee) to have stabilised. This will be completed at the investigator’s (or designee’s) discretion.

7.2.2. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations at the times indicated in the Schedule of Assessments in Appendix 6. Clinical laboratory evaluations are listed in Appendix 2. The timing of clinical laboratory evaluations may be adjusted and/or additional clinical laboratory evaluations may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of clinical laboratory safety evaluations is required.

Subjects will be asked to provide urine samples for a drugs of abuse screen and cotinine test, and will undergo an alcohol breath test at the times indicated in the Schedule of Assessments

in Appendix 6. For all female subjects, a pregnancy test will be performed at the times indicated in the Schedule of Assessments in Appendix 6.

An investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

7.2.3. Vital Signs

Supine blood pressure, supine pulse rate, respiratory rate, and oral body temperature will be assessed at the times indicated in the Schedule of Assessments in Appendix 6. Vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

Day 1 predose blood pressure, pulse rate, and respiratory rate will be measured in triplicate at approximately 2-minute intervals. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges. Oral body temperature will be measured singly.

Subjects must be supine for at least 5 minutes before blood pressure and pulse rate measurements.

In the event orthostatic measurements are required (if deemed clinically appropriate by an investigator or designee), the supine blood pressure and pulse rate will be measured after the subject has been supine for at least 5 minutes. The subject will then stand for at least 2 minutes and the standing blood pressure and pulse rate will be measured.

7.2.4. Electrocardiogram

7.2.4.1. 12-lead Electrocardiogram

Resting 12-lead ECGs will be recorded after the subject has been supine and at rest for at least 5 minutes at the times indicated in the Schedule of Assessments in Appendix 6. Single 12-lead ECGs will be repeated once if either of the following criteria apply:

- QTcF value > 500 ms
- QTcF change from the baseline (predose) is > 60 ms.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of ECGs is required. The investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

Day 1 predose 12-lead ECGs will be measured in triplicate at approximately 2-minute intervals. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges.

7.2.4.2. Telemetry

Cardiac rhythm will be monitored by telemetry at the times indicated in the Schedule of Assessments in Appendix 6.

Telemetry is not planned for Part B of the study but may be implemented if any clinically significant findings are identified in the data from Part A.

7.2.4.3. Continuous 12-lead Electrocardiogram Monitoring

Continuous 12-lead ECG monitoring using a digital recorder will take place at the times indicated in the Schedule of Assessments in Appendix 6. Continuous 12-lead ECG monitoring may be added to Part B; if added, the details will be documented and filed in the TMF.

All continuous 12-lead ECG data collected will be archived without extraction or analysis and will not be reported in the scope of this study.

Subjects will be supine for at least 10 minutes before the extraction timepoint and for 5 minutes from the start of each extraction timepoint (each extraction will last for 5 minutes). Environmental distractions (eg, television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording.

When coinciding, vital signs assessments and PK sampling should always be performed after the ECG extraction time window. If a separate ECG machine is being used for safety assessments described in Section 7.2.4.1, that machine should be in place prior to the extraction window to permit safety ECGs to be recorded irrespective of the extraction window. If the machine is not in place prior to the extraction window, safety ECGs must be recorded after the extraction window. If an integral system is used, safety ECGs may be recorded irrespective of the extraction window.

7.2.5. Physical Examination

A full physical examination will be performed at screening and follow-up, and a symptom-directed physical examination will be performed at all other timepoints specified in the Schedule of Assessments in Appendix 6.

7.2.6. Body Weight

Body weight (in underclothes) will be recorded at the times indicated in the Schedule of Assessments in Appendix 6.

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

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.

8.2. Analysis Populations

8.2.1. 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 quantifiable PK concentration.

8.2.2. Safety Population

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

8.3. Pharmacokinetic Analyses

Non-compartmental PK analysis will be performed on individual plasma and urine concentration data, using commercial software such as Phoenix® WinNonlin®.

The PK concentrations and parameters will be listed and summarised using descriptive statistics.A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} on Day 1 in Part A and $AUC_{0\text{-}\tau}$ and C_{max} on Day 14 in Part B. The PK parameters will be analysed using a power model. However, if the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be natural log-transformed and analysed using an analysis of variance model.

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 median time to maximum concentration.

8.4. Safety Analysis

Safety parameters will be listed and summarised using descriptive statistics. No formal statistical analysis of safety data is planned. Each AE will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

If conducted, the results from the cardiodynamic ECG assessment will be reported separately. Further detail of the analyses will be covered by the standard operating procedures (SOPs) and/or the analysis plan of the cardiac vendor.

8.5. Interim Analysis

No formal interim analyses are planned for this study.

9. REFERENCES

1. NorthSea Therapeutics B.V. NST-6179 – Investigator's Brochure. (Version 1.0). 09 March 2021.

2. Food and Drug Administration. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

[Internet]. Food and Drug Administration; 2005. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf

10. APPENDICES

Appendix 1: Adverse Event Reporting

Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and/or unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a study treatment, whether or not related to the study treatment.

Assessment of Severity

The investigator will be asked to provide an assessment of the severity of the AE using the following categories:

- **Mild:** Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate:** Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- **Severe:** Interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Relationship to Study Treatment

The investigator (or designee) will make a determination of the relationship of the AE to the study treatment using a 2-category or binary approach system according to the following guidelines:

- **Not Related:** The AE is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Related:** The AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesised cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

Follow-up of Adverse Events

Every reasonable effort will be made to follow up with subjects who have AEs. Any subject who has an ongoing AE that is related to the investigational medicinal product (IMP) or study procedures at the follow-up visit will be followed up, where possible, until resolution or until the unresolved AE is judged by the investigator (or designee) to have stabilised. This will be completed at the investigator's (or designee's) discretion. Any subject who has an ongoing AE that is not related to the IMP or study procedures at the follow-up visit can be closed out as ongoing at the investigator's discretion.

Adverse Drug Reactions

All noxious and unintended responses to an IMP (ie, where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg, Investigator's Brochure [IB] for an unapproved IMP).

Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose either:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- results in a congenital anomaly/birth defect
- results in an important medical event (see below).

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered SAEs when, based upon appropriate medical judgement, they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Instances of death or congenital abnormality, if brought to the attention of the investigator at any time after cessation of the study treatment and considered by the investigator to be related to the study treatment, will be reported to the sponsor.

Definition of Life-threatening

An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred (ie, does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

Definition of Hospitalisation

Adverse events requiring hospitalisation should be considered serious. In general, hospitalisation signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the study site. When in doubt as to whether hospitalisation occurred or was necessary, the AE should be considered as serious.

Hospitalisation for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AEs and should be recorded on a clinical assessment form and added to the electronic case report form (eCRF). If anything untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'non-serious' attributed according to the usual criteria.

Serious Adverse Event Reporting

Emas Pharma Limited, Trading as Bionical Emas, Hertfordshire, United Kingdom is responsible for coordinating the reporting of SAEs in accordance with the European Directive 2001/20/EC.

The investigator will complete an SAE report form and forward it by email (drug.safety@bionical-emas.com) to Emas Pharma Limited and the sponsor immediately (within 24 hours) upon becoming aware of an SAE.

The responsibilities of Emas Pharma Limited include the following:

- Prepare an SAE Management Plan prior to the start of the study. Where this plan differs from the applicable Clinical Research Unit (CRU) standard operating procedure (SOP) on SAE reporting, the SAE Management Plan will always take precedence.
- Receive and review SAE report forms from the CRU and inform the sponsor of the SAE within 1 working day of the initial notification to Emas Pharma Limited. Emas Pharma Limited will delete any information from the SAE report forms that may identify the subject.
- Write case narratives and enter the case into the safety database as defined in the SAE Management Plan.
- Produce appropriate reports of all suspected unexpected serious adverse reactions and forward to the institutional review board (IRB)/ethics committee (EC), MHRA, investigator, and the sponsor within the timeframes stipulated in the Clinical Trials Directive Guideline (ENTR/CT 3).

The responsibility for reporting SAEs will be transferred to the sponsor 28 days after the end of the study.

Pregnancy

Pregnancy (maternal or paternal exposure to study treatment) does not meet the definition of an AE. However, to fulfil regulatory requirements any pregnancy should be reported by the investigator to Emas Pharma Limited and the sponsor immediately (within 24 hours) upon becoming aware of the pregnancy, by completing a Pregnancy report form and forwarding by email to drug.safety@bionical-emas.com. The pregnancy will be followed up to collect data on the outcome for both mother and foetus.

Appendix 2: Clinical Laboratory Evaluations

Clinical chemistry:	Haematology:	Urinalysis:
Alanine aminotransferase (ALT) Albumin Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) Calcium Chloride Cholesterol Creatinine Direct bilirubin Gamma-glutamyl transferase (GGT) Glucose Inorganic phosphate Potassium Sodium Total bilirubin Total CO_2 Total protein Uric acid	Haematocrit Haemoglobin Mean cell haemoglobin Mean cell haemoglobin concentration Mean cell volume Platelet count Red blood cell (RBC) count White blood cell (WBC) count WBC differential: Basophils Eosinophils Lymphocytes Monocytes Neutrophils	Blood Glucose Ketones pH Protein Specific gravity Urobilinogen Microscopic examination
Serology:[a]	**Drug screen:**	**Coagulation profile:**
Anti-hepatitis B surface antibody Hepatitis B surface antigen (HBsAg) Hepatitis C antibody Human immunodeficiency virus (HIV-1 and HIV-2) antibodies and p24 antigen	Including but not limited to: Amphetamines/methamphetamines Barbiturates Benzodiazepines Cocaine (metabolite) Methadone Phencyclidine Opiates Tetrahydrocannabinol/cannabinoids Tricyclic antidepressants Cotinine test	Activated partial thromboplastin time (aPTT) International normalized ratio (INR) Prothrombin time (PT)
		Hormone panel - females only:
		Follicle-stimulating hormone (FSH)[a] Serum pregnancy test (human chorionic gonadotropin)[b] Urine pregnancy test[b]
		Fasting Lipid Panel (Cohorts B4 onwards):
		Low-density lipoprotein cholesterol (LDL-c) High-density lipoprotein cholesterol (HDL-c) Very low-density lipoprotein (VLDL) Total Cholesterol Triglycerides (TG) Apolipoprotein (Apo) A1 ApoB ApoC3

a. Performed at screening only.
b. For all female subjects, performed in serum at screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.

Appendix 3: Total Blood Volume

Part A

The following blood volumes will be withdrawn for each subject:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	5	37.5
Serology	3.5	1	3.5
Coagulation	1.8	5	9.0
NST-6179 pharmacokinetics[b]	2.0	19	38.0
Pharmacogenomics[c]	6.0	1	6.0
Total:			94.0

Abbreviation: FSH = follicle-stimulating hormone.
a. Includes pregnancy and FSH tests.
b. Includes 3 additional samples that may be taken.
c. This is an optional sample, which may be collected with the subject's consent.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 140 mL.

Part B

The following blood volumes will be withdrawn for each subject not participating in a group with metabolite identification:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	7	52.5
Serology	3.5	1	3.5
Coagulation	1.8	7	12.6
NST-6179 pharmacokinetics[b]	2.0	35	70.0
Pharmacogenomics[c]	6.0	1	6.0
Fasting lipid panel	2.5	3	7.5
Total:			152.1

Note: Increased dosing frequency may require 2 to 3 additional PK sample collections, however, the maximum blood volume collected will not exceed 190 mL.
Abbreviation: FSH = follicle-stimulating hormone; PK = pharmacokinetic(s).
a. Includes pregnancy and FSH tests.
b. Includes 3 additional samples (per PK sampling day) that may be taken.
c. This is an optional sample, which may be collected with the subject's consent.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 190 mL.

The following blood volumes will be withdrawn for each subject participating in a group with metabolite identification:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	7	52.5
Serology	3.5	1	3.5
Coagulation	1.8	7	12.6
NST-6179 pharmacokinetics[b]	2.0	35	70.0
Metabolite identification[b]	2.0	35	70.0
Pharmacogenomics[c]	6.0	1	6.0
Total:			214.6

Abbreviation: FSH = follicle-stimulating hormone; PK = pharmacokinetic(s).
a. Includes pregnancy and FSH tests.
b. Includes 3 additional samples (per PK sampling day) that may be taken.
c. This is an optional sample, which may be collected with the subject's consent.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 270 mL.

Appendix 4: Contraception Guidance

Definitions

Female of Childbearing Potential: premenopausal female who is anatomically and physiologically capable of becoming pregnant following menarche.

Female of Non-childbearing Potential:

1. **Surgically sterile:** female who is permanently sterile via hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy by reported medical history and/or medical records. Surgical sterilisation to have occurred a minimum of 6 weeks, or at the investigator's discretion, prior to screening.
2. **Postmenopausal:** females at least 45 years of age with amenorrhea for 12 months without an alternative medical reason with confirmatory follicle-stimulating hormone (FSH) level of ≥ 40 mIU/mL. The amenorrhea should not be induced by a medical condition such as anorexia nervosa, hypothyroid disease or polycystic ovarian disease, or by extreme exercise. It should not be due to concomitant medications that may have induced the amenorrhea such as oral contraceptives, hormones, gonadotropin-releasing hormones, anti-oestrogens, or selective oestrogen receptor modulators. Females on hormone replacement therapy with FSH levels < 40 mIU/mL may be included at the discretion of the investigator. Females aged > 60 years old whose FSH values are not ≥ 40 mIU/mL may be included at the discretion of the investigator and in consultation with the sponsor.

Fertile male: a male that is considered fertile after puberty.

Infertile male: permanently sterile male via bilateral orchiectomy.

Contraception Guidance

Female Subjects

Female subjects who are of non-childbearing potential will not be required to use contraception. Female subjects of childbearing potential must be willing to use 2 methods (1 primary and 1 secondary method) of birth control from the time of signing the informed consent form (ICF) until 90 days after the follow-up visit. Primary (non-barrier) methods of contraception include:

- hormonal injection (as prescribed)
- combined oral contraceptive pill or progestin/progestogen-only pill (as prescribed)
- combined hormonal patch (as prescribed)
- combined hormonal vaginal ring (as prescribed)
- surgical method performed at least 3 months prior to the screening visit:
    - bilateral tubal ligation

    - Essure® (hysteroscopic bilateral tubal occlusion) with confirmation of occlusion of the fallopian tubes
- hormonal implant
- hormonal or non-hormonal intrauterine device (IUD)
- vasectomised male partner (sterilisation performed at least 90 days prior to the screening visit, with verbal confirmation of surgical success, and the sole partner for the female subject).

Secondary (barrier) methods of contraception include:

- male condom with spermicide
- female condom with spermicide
- over-the-counter sponge with spermicide
- cervical cap with spermicide (as prescribed)
- diaphragm with spermicide (as prescribed).

Female subjects of childbearing potential should refrain from donation of ova from check-in (Day -1) until 90 days after the follow-up visit.

Male Subjects

Male subjects (even with a history of vasectomy) with partners of childbearing potential must use a male barrier method of contraception (ie, male condom with spermicide) in addition to a second method of acceptable contraception from check-in until 90 days after the follow-up visit. Acceptable methods of contraception for female partners include:

- hormonal injection
- combined oral contraceptive pill or progestin/progestogen-only pill
- combined hormonal patch
- combined hormonal vaginal ring
- surgical method (bilateral tubal ligation or Essure® [hysteroscopic bilateral tubal occlusion])
- hormonal implant
- hormonal or non-hormonal IUD
- over-the-counter sponge with spermicide
- cervical cap with spermicide
- diaphragm with spermicide.

An acceptable second method of contraception for male subjects is vasectomy that has been performed at least 90 days prior to the screening visit, with verbal confirmation of surgical success.

For male subjects (even with a history of vasectomy), sexual intercourse with female partners who are pregnant or breastfeeding should be avoided unless condoms are used from the time of the first dose until 90 days after the follow-up visit. Male subjects are required to refrain from donation of sperm from check-in until 90 days after the follow-up visit.

Sexual Abstinence and Same-sex Relationships

Subjects who practice true abstinence, because of the subject's lifestyle choice (ie, the subject should not become abstinent just for the purpose of study participation), are exempt from contraceptive requirements. Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception. If a subject who is abstinent at the time of signing the ICF becomes sexually active, they must agree to use contraception as described previously.

For subjects who are exclusively in same-sex relationships, contraceptive requirements do not apply. If a subject who is in a same-sex relationship at the time of signing the ICF becomes engaged in a heterosexual relationship, they must agree to use contraception as described previously.

Appendix 5: Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
- Applicable International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
- Applicable laws and regulations.

The protocol, protocol amendments, informed consent form (ICF), Investigator's Brochure (IB), and other relevant documents must be submitted to an institutional review board (IRB)/ethics committee (EC) by the investigator and reviewed and approved by the IRB/EC before the study is initiated.

Any substantial protocol amendments, likely to affect the safety of the subjects or the conduct of the study, will require IRB/EC and regulatory authority (as locally required) approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects or any non-substantial changes, as defined by regulatory requirements.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC.
- Notifying the IRB/EC of serious adverse events (SAEs) or other significant safety findings as required by IRB/EC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/EC, European Directive 2001/20/EC for clinical studies (if applicable), and all other applicable local regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each subject will be provided with a study-specific ICF giving details of the study treatments, procedures, and potential risks of the study. Subjects will be instructed that they are free to obtain further information from the investigator (or designee) and that their participation is voluntary and they are free to

withdraw from the study at any time. Subjects will be given an opportunity to ask questions about the study prior to providing consent for participation.

Following discussion of the study with study site personnel, subjects will sign the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. A copy of the ICF will be given to the subject.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

Subject Data Protection

Subjects will be assigned a unique identifier and will not be identified by name in electronic case report forms (eCRFs), study-related forms, study reports, or any related publications. Subject and investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual subjects or investigators will be redacted according to applicable laws and regulations.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must also be informed that his/her study-related data may be examined by sponsor or contract research organisation (CRO) auditors or other authorised personnel appointed by the sponsor, by appropriate IRB/EC members, and by inspectors from regulatory authorities.

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The investigator (or designee) agrees not to disclose such information in any way without prior written permission from the sponsor.

Data Quality Assurance

The following data quality steps will be implemented:

- All relevant subject data relating to the study will be recorded on eCRFs unless directly transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB/EC review, and regulatory agency inspections and provide direct access to source data documents.
- Labcorp Drug Development is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds,

quality tolerance thresholds, controls, and mitigation plans will be documented in a project management plan. Additional details of quality checking to be performed on the data may be included in a data management plan.

- A study monitor will perform ongoing source data verification to confirm that data entered into the eCRF by authorised site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator in the study site archive for at least 5 years after the end of the study unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Investigator Documentation Responsibilities

All individual, subject-specific study data will also be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion.

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to Labcorp Drug Development electronically, will be integrated with the subject's eCRF data in accordance with the data management plan.

An eCRF must be completed for each enrolled subject who undergoes any screening procedures, according to the eCRF completion instructions. The sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the investigator reviewed and approved the data on the eCRF, data queries, and site notifications.

Publications

Publications will be addressed in a separate agreement. The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicentre studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Appendix 6: Schedule of Assessments

Schedule of Assessments – Part A (Single Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Treatment Period 1		Follow-up (10 to 14 days postdose)
		Day -1	Days 1 to 4	
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographic data	X			
Medical history	X	X[a]		
Urinary drug screen	X	X		
Alcohol breath test	X	X		
Urine cotinine test	X	X		
Serology	X			
Pregnancy test[b]	X	X		X
FSH[c]	X			
Height and body weight	X[d]	X		
Study residency:				
Check-in		X		
Check-out			Day 4	
Non-residential visit	X			X
Study treatment administration:				
NST-6179 or placebo			Day 1 (0 hour)	
Pharmacokinetics:				
Blood sampling			Predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours postdose	X
Urine sampling			Predose (spot collection) and 0 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 48, and 48 to 72 hours postdose	
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X

Schedule of Assessments – Part A (Single Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Treatment Period 1		Follow-up (10 to 14 days postdose)
		Day -1	Days 1 to 4	
Clinical chemistry, haematology, and urinalysis	X	X	Predose and 48 hours postdose	X
Coagulation	X	X	Predose and 48 hours postdose	X
Blood pressure, pulse rate, respiratory rate, and oral body temperature	X		Predose,[e] and 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 hours postdose	X
12-lead ECG	X		Predose,[e] and 2, 4, 12, 24, 48, and 72 hours postdose	X
Telemetry			From at least -1 hour predose to 4 hours postdose	
Continuous 12-lead ECG[f]			3 (triplicate) continuous 12-lead extractions taken prior to dosing, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours postdose	
Full physical examination	X[g]			X
Symptom-directed physical examination			Prior to discharge on Day 4	
Pharmacogenomics blood sample			Predose[h]	

Abbreviations: ECG = electrocardiogram; FSH = follicle-stimulating hormone.

a. Interim medical history.
b. Performed in serum at screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.
c. Performed in all female subjects (to confirm postmenopausal status).
d. Height measured at screening only.
e. Predose assessments will take place within 2 hours prior to dosing.
f. Monitor for 12-lead ECG recording will be worn from 2 hours predose to 25 hours postdose on Day 1. Prior to dosing, 3 (ie, triplicate) continuous 12-lead extractions are required. The predose extraction timepoints will be averaged for baseline.
g. Performed between the screening visit and predose assessments.
h. Optional sample; this sample is not time-sensitive, if not possible to collect predose on Day 1, it can be collected at any time during or after the course of the study.

Schedule of Assessments – Part B (Multiple Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Day -1	Days 1 to 16	Follow-up (10 to 14 days post-final dose)
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographic data	X			
Medical history	X	X[a]		
Urinary drug screen	X	X		
Alcohol breath test	X	X		
Urine cotinine test	X	X		
Serology	X			
Pregnancy test[b]	X	X		X
FSH[c]	X			
Height and body weight	X[d]	X		
Study residency:				
Check-in		X		
Check-out			Day 16 (48 hours post-final dose)	
Non-residential visit	X			X
Study treatment administration:				
NST-6179 or placebo			QD dosing: Day 1 to 14 (0 hour) BID dosing:[g] Days 1 to 13 BID (0 hour [AM dose] and 12±0.5 hours later [PM dose]); Day 14 (0 hour [AM dose] only)	

Schedule of Assessments – Part B (Multiple Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Day -1	Days 1 to 16	Follow-up (10 to 14 days post-final dose)
Pharmacokinetics:				
Blood sampling			QD dosing: Day 1: predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours postdose Days 2, 4, 6, 8, 10, and 12: predose Day 14: predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose BID dosing: Day 1: pre-AM dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours postdose (pre-PM dose) Days 2, 4, 6, 8, and 10: pre-AM dose Day 12 and 13: pre-AM dose and pre-PM dose Day 14: pre-AM dose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours postdose	
Urine sampling (PK for all dose groups and metabolite identification for highest planned QD dose group only)			QD dosing: Day 1: predose (spot collection) and 0 to 4, 4 to 8, 8 to 12, 12 to 24 hours postdose Day 14: predose (spot collection) and 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours postdose BID dosing: Day 1: pre-AM dose (spot collection) and 0 to 4, 4 to 8, and 8 to 12 hours postdose (pre-PM dose) Day 14: pre-AM dose (spot collection) and 0 to 4, 4 to 8, 8 to 12, 12 to 24, and 24 to 48 hours postdose	

Schedule of Assessments – Part B (Multiple Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Day -1	Days 1 to 16	Follow-up (10 to 14 days post-final dose)
Blood sampling for metabolite identification (for highest planned dose group only)			QD dosing only: Day 1: predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours postdose Days 2, 4, 6, 8, 10, and 12: predose Day 14: predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours postdose	
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X
Clinical chemistry, haematology, and urinalysis	X	X	Days 1, 4, and 8: pre-AM dose Day 14: 48 hours postdose	X
Coagulation	X	X	Days 1, 4, and 8: pre-AM dose Day 14: 48 hours postdose	X
Fasting lipid panel[h]			Days 1, 7, and 14: pre-AM dose	
Blood pressure, pulse rate, respiratory rate, and oral body temperature	X		QD dosing: Day 1: predose[e], and 1, 2, 4, and 12 hours postdose Days 2 to 14: predose and 4 hours postdose BID dosing: Day 1: pre-AM dose and 1, 2, 4, and 12 hours postdose (pre-PM dose) Days 2 to 14: pre-AM dose (0 hour) and 4 hours postdose	X

Schedule of Assessments – Part B (Multiple Dose in Healthy Subjects)

Study Procedures	Screening (Day -28 to Day -2)	Day -1	Days 1 to 16	Follow-up (10 to 14 days post-final dose)
12-lead ECG	X		QD dosing: Day 1: predose[e], and 2, 4, and 12 hours postdose Days 4, 7, and 10: predose Day 14: predose, 2, 4, 12, 24, and 48 hours postdose BID dosing: Day 1: pre-AM dose and 2, 4, and 12 hours postdose (pre PM dose) Day 4, 7, and 10: pre-AM dose Day 14: pre-AM dose and 2, 4, 12, 24, and 48 hours postdose	X
Full physical examination	X[f]			X
Symptom-directed physical examination			Prior to discharge on Day 16	
Pharmacogenomics blood sample			Predose[i]	

Abbreviations: AM = morning; ECG = electrocardiogram; FSH = follicle-stimulating hormone; PK = pharmacokinetic(s); PM = evening.

a. Interim medical history.
b. Performed in serum at screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.
c. Performed in all female subjects (to confirm postmenopausal status).
d. Height measured at screening only.
e. Predose assessments will take place within 2 hours prior to dosing.
f. Performed between the screening visit and predose assessments.
g. For BID dosing, the AM and PM dosing time for each subject will be as consistent as possible for each day.
h. Only for Cohorts B4 onwards.
i. Optional sample; this sample is not time-sensitive, if not possible to collect predose on Day 1, it can be collected at any time during or after the course of the study.

Appendix 7: Summary of Amended Protocol Changes

Version 4 to Version 5

Major changes:

1. Rationale for protocol amendment: This is a new section added to details the reasons this amendment has been issued.
2. Section 1.6.1: This is a new section added to include relevant data from previously completed cohorts. This section includes subsections for clinical pharmacokinetic (Section 1.6.1.1) and clinical safety data (Section 1.6.1.2).
3. Sections 2.1 and 2.2.3: Exploratory objective and endpoint added for the evaluation of the effect of pharmacogenomics on the pharmacokinetics of NST-6179.
4. Sections 2.2.3, 7.1.2: Clarified that additional samples for metabolite identification will be collected for the highest planned QD dose group.
5. Sections 3.1.2, 3.3, 3.4, and 3.5: Dosing restriction was revised from an exposure basis to an established safe dose level basis, ie, the dose of NST-6179 administered on each dosing occasion in Part B will not exceed a single dose previously shown to be safe and well tolerated in Part A. Clarified in the footnotes for Figures 3 and 4 that the dosing frequency and duration in Part B may be change from the planned QD dosing.
6. Section 3.5: Table 7, previously Table 6, updated to present the doses which have already been administered in Parts A and B.
7. Sections 3.1.2, 3.4, 3.4.1, and 5.3:To enhance subject safety, a requirement for sentinel dosing for groups in Part B for which the total daily dose exceeds the highest single dose evaluated in Part A was added.
8. Section 5.2: Details for administration of a BID dosing regimen have been added.
9. Section 5.2 and 6.2: Details for the administration of a BID dosing regimen under fasted conditions have been added.
10. Section 7.1.4: This is a new section added to include the collection of an optional pharmacogenomics sample.
11. Appendix 2: Fasting lipid panel parameters were added to Part B for Cohorts B4 onwards.
12. Appendix 3: The total blood volumes drawn for Part A were updated to include an additional sample for pharmacogenomics. The total blood volumes drawn for Part B, for both subjects participating and not participating in metabolite identification, were updated to include additional samples for pharmacogenomics. The total blood volumes drawn for subjects not participating in metabolite identification in Part B, has been updated to include samples for the fasting lipid panel.
13. Appendix 6, Schedule of Assessment for Part A (Single Dose in Healthy Subjects): Added a row for the collection of an optional pharmacogenomics blood sample predose on Day 1, an associated footnote (h) was added to clarify that this sample could be collected at any time during or after the study.

14. Appendix 6, Schedule of Assessment for Part B (Multiple Dose in Healthy Subjects): Study procedures were modified in support of BID dosing as follows:
    a. Added BID dosing to study treatment administration, 12±0.5 hours apart, starting in the morning (AM) of Day 1, completing dosing with an AM dose on Day 14. The AM and PM dosing time for each subject will be as consistent as possible for each day.
    b. Pharmacokinetic blood sampling modified to add trough collections pre-PM dose on Days 12 and 13 and a 16 hour postdose sample on Day 14. Added clarification that predose collections will take place pre-AM dose and the 12 hours postdose collection on Day 1 is to be collected pre-PM dose to enable these collections to reflect single dose analysis as for Part B QD dose groups.
    c. Urine sampling modified to remove the 12 to 24 hour postdose collection interval on Day 1 because this interval will be after the PM dose on Day 1, and therefore no longer relevant for comparison with Part B QD dose groups. Clarified that predose collections will take place pre-AM dose. Additionally, clarified that urine samples for metabolite identification will be collected for the highest planned QD dose group.
    d. Blood samples for metabolite identification samples will be collected for Part B QD dose groups only.
    e. Added clarification that coagulation, clinical chemistry, haematology, and urinalysis predose samples will be collected pre-AM dose to remain comparable to Part B QD dose groups.
    f. Added clarification that the vital signs and ECG predose assessments will be pre-AM dose and the 12-hour postdose assessments on Day 1 are to be performed pre-PM dose to remain comparable to Part B QD dose groups.
    g. Added a row for a fasting lipid panel assessment on Days 1, 7, and 14, an associated footnote (h) was added to clarify this assessment is only applicable to Cohorts B4 onwards.
    h. Added a row for the collection of an optional pharmacogenomics blood sample predose on Day 1, an associated footnote (i) was added to clarify that this sample could be collected at any time during or after the study.

Minor changes:

1. Added PK parameter specific to BID dosing (area under the plasma concentration time curve (AUC) from time zero to 24 hours postdose (AUC_{0-24}, derived as $AUC_{0-\tau}$*2 for a BID dosing regimen at steady state).
2. A footnote was added to the blood volume tables for Part B in Appendix 3, noting that additional PK blood sample collections may be required due to increased dosing frequency but the maximum blood volume collected would not exceed 450 mL.
3. The synopsis was updated according to changes in the protocol body, as applicable.

4. The amendment/version number and date were updated throughout the protocol.
5. Abbreviations were added and/or removed, as appropriate.
6. Minor editorial updates were made.

Version 3 to Version 4

Major changes:

1. Text in Section 1.4.3 amended to update the findings of the 13-week oral (gavage) toxicity and toxicokinetic study in the monkey with a 2-week recovery period (Covance study 8423746).
2. Section 1.4.4 is a new section added to present the current results of the ongoing 13-week oral (gavage) administration toxicity study in the mouse (Labcorp study 8423764).
3. Section 1.8 updated to clarify the risk-benefit of conducting this first-in-human study (NST-6179-001) remains unchanged and the proposed stopping rules remain supported by data from the mouse, rat, and monkey.
4. Removal of food-effect cohort from Part A, and clarification that all doses in Part B will be administered in the fasted state. The way the molecule should not be affected significantly by food and so a food-effect evaluation is not considered necessary.
5. Appendix 6, Schedule of Assessment for Part A updated to clarify the clinical chemistry, haematology, urinalysis, and coagulation assessment will be at 48 hours postdose (previously 72 hours postdose).

Minor changes:

6. The synopsis was updated according to changes in the protocol body, as applicable.
7. The amendment/version number and date were updated throughout the protocol.
8. The header was updated to reflect Covance being rebranded as Labcorp.
9. Abbreviations were added and/or removed, as appropriate.
10. Minor editorial updates were made.

A detailed summary of changes is presented below:

For each section, deleted text is shown in ~~strikethrough~~, and new text is shown in **bold**.

Section 1.4.3: 13-week Oral (Gavage) Toxicity and Toxicokinetic Study in the Monkey with a 2-week Recovery Period

Previously read:

Animals were dosed through Day 15 and ~~was~~ NST-6179 generally well tolerated with only mild spurious vomiting and an 8% decrease in bodyweight observed in the early stages, both of which returned to baseline status over time. No other relevant clinical findings have been observed to date at any dose level in either male or female monkeys. However, on Day 16~~,~~ a

single female monkey ~~in the high dose group~~ was found dead following dosing on Day 15. The decedent female appeared to be in general good health and displayed no clinical signs up through and postdose on Day 15. ~~Dosing was temporarily held in the high dose female cohort and out of caution was restarted at a lower dose of 300 mg/kg/day and the frequency of postdose monitoring was increased.~~ A macroscopic post-mortem examination showed no advanced autolysis at macroscopic post-mortem indicat~~ed~~ the female died relatively soon prior to the post-mortem examination~~. Although an exact time of death cannot be established, the relative time frame of death was close to 24 hours after the Day 15 dose,~~ when minimal drug concentrations levels would have been present. Microscopically, minimal alveolar/interstitial inflammation was noted ~~which is~~ consistent with post aspiration effects~~. However, the minimal severity and distribution is considered~~ not ~~to be~~ the cause of mortality. The majority of tissues were well preserved ~~but~~ some autolysis ~~was~~ noted in the gall bladder and gastrointestinal tract and to a lesser extent liver, pancreas and salivary glands. ~~Further detailed examination of the clinical condition and study data from the decedent animal was also reviewed to determine any underlying~~ condition ~~existed~~ as a potential cause of death. Clinical chemistry and urinalysis parameters were all normal. ~~Haematology parameters were all within normal limits, with the exception of minimal increases in white cells and absolute neutrophil count. However, no evidence of infection was noted associated with these elevations. A minimal decrease in red cells, haemoglobin and packed cell volume was noted. None of these changes were felt to be clinically meaningful nor contributory to the cause of death.~~ Assessment of the decedents ECG indicated minor anomalies considered not clinically relevant.

A review of the data from the prior 28-day repeat dose oral toxicity study in monkeys established 350 mg/kg/day was the NOAEL level (Covance 8423746). No clinical signs and symptoms or mortality ~~was~~ observed at this dose level. Importantly, in the ~~ongoing~~ 13-week study NST-6179 ~~continues to be~~ well tolerated in all other males and females ~~at all dosages up to 28 days of dosing~~. In ~~summary~~, ~~all of the clinical and laboratory data preceding the event, as well as the post-mortem assessments, have been peer reviewed by two pathologists, both of whom concur with the observations and the lack of evidence of a drug-related event~~.

Now reads:

Animals were dosed through Day 15 and NST-6179 **was** generally well tolerated with only mild spurious vomiting and an 8% decrease in bodyweight observed in the early stages, both of which returned to baseline status over time. No other relevant clinical findings have been observed to date at any dose level in either male or female monkeys. However, **there were subsequently 2 events of note in the high-dose cohort (350 mg/kg/day). The first event occurred** on Day 16 **in which** a single female monkey was found dead following dosing on Day 15. The decedent female appeared to be in general good health and displayed no clinical signs up through and postdose on Day 15. A macroscopic post-mortem examination showed no advanced autolysis at macroscopic post-mortem indicat**ing** the female died relatively soon prior to the post-mortem examination **and at a timeframe** when minimal drug concentrations levels would have been present. Microscopically, minimal alveolar/interstitial inflammation was noted**,** consistent with post aspiration effects **but** not the cause of mortality. The majority of tissues were well preserved **with** some autolysis noted in the gall bladder and gastrointestinal tract and to a lesser extent liver, pancreas**,** and salivary glands. **No pre-existing comorbid** condition**s were identified** as a potential cause of death. Clinical

chemistry and urinalysis parameters were all normal **or with minimal changes; none of which were felt to be clinically relevant.** Assessment of the decedents ECG indicated minor anomalies considered not clinically relevant. **As of this time, there is no clear indication of the cause of death but the examinations to date do not show any evidence of direct tissue injury or toxicity associated with NST-6179.**

The second event occurred in the high-dose cohort (350 mg/kg/day) where a male monkey was found in a moribund condition 4 hours after dosing and euthanized on Day 87. This male was in good health throughout the study and prior to the last dose on Day 87. The investigation into the cause of death is still ongoing as well as an assessment of the remaining animals in the high-dose cohort. However, the initial assessment from the study pathologist determined that the microscopic findings were common background changes in this strain of monkey. However, there was moderate bilateral tubular nephropathy in the kidney which could be possibly related to the cause of death but will be confirmed with further investigations of this animal and the others in the cohort. No other clinical or laboratory findings revealed any clear signs of drug toxicity that would lead to death.

A review of the data from the prior 28-day repeat dose oral toxicity study in monkeys established 350 mg/kg/day was the NOAEL level (Covance 8423746). No clinical signs and symptoms or mortality **were** observed at this dose level. Importantly, **dosing** in the **monkey** 13-week study **is now complete.** NST-6179 **was** well tolerated in all other **surviving** male and female **high dose monkeys. The low (60 mg/kg/day) and medium (210 mg/kg/day) doses were well-tolerated throughout the study period with no notable events. Both of the events in the high-dose group were at exposures far exceeding the exposure of the likely doses in the Phase 1 study.** In **addition, there was no clear drug-related effect with the first monkey and the second event occurred at a duration much longer than the maximal 14-day duration in the Phase 1 study.**

Section 1.4.4 (new section): 13-week Oral (Gavage) Administration Toxicity Study in the Mouse (Labcorp Study Number 8423764; ongoing)

A 13-week oral toxicity study in the mouse conducted under GLP is currently ongoing to determine dose level selection in a subsequent 2-year carcinogenicity study as well as evaluating TK at Day 1 and Day 90 of the study.

The maximum dose levels administered in the 13-week study were based on a previous mouse dose range finding study which was conducted to establish the maximum tolerated oral dose (MTD) in male and female mice. This study established an MTD of 250 mg/kg in female mice and an MTD of 200 mg/kg/day in male mice. These dose levels were tolerated for 21 days without mortality or adverse clinical signs. The selected low dose level of 70 mg/kg/day is the approximate effective dose. The intermediate dose levels in males and females represent the mid-point between 70 mg/kg/day and 200 mg/kg/day (males) and 250 mg/kg/day (females).

Group assignment is indicated in Table 1**.**

Table 1: Group Assignment in the 13-week Oral (Gavage) Administration Toxicity Study in the Mouse (Labcorp Study Number 8423764)

Group[a]	Subgroup	Dose Level[b] (mg/kg/day)	Dose Concentration[b] (mg/mL)	Number of Animals	
				Males	Females
1 (Control)	1 (Toxicity)	0	0	12	12
	2 (Toxicokinetic)	0	0	3	3
2 (Low)	1 (Toxicity)	70	7	12	-
	1 (Toxicity)	70	7	-	12
	2 (Toxicokinetic)	70	7	18	-
	2 (Toxicokinetic)	70	7	-	18
3 (Intermediate)	1 (Toxicity)	135	13.5	12	-
	1 (Toxicity)	160	16	-	12
	2 (Toxicokinetic)	135	13.5	18	-
	2 (Toxicokinetic)	160	16	-	18
4 (High)	1 (Toxicity)	200	20	12	-
	1 (Toxicity)	250	25	-	12
	2 (Toxicokinetic)	200	20	18	-
	2 (Toxicokinetic)	250	25	-	18

a. Group 1 administered vehicle control only (corn oil).
b. Animals dosed at a volume of 10 mL/kg.

On Day 1 of dosing in the 13-week study all mice were dosed by oral gavage at the doses outlined above. No adverse effects were observed at the low and intermediate dose levels. At the high dose levels, approximately 22 hours after the first dose, 1 male and 2 females were found dead. Additionally, 1 female displayed clinical signs of ataxia, reduced activity, hunched posture, and tremors leading to the animal being euthanized. Eight days after the first dose, 1 female was euthanized due to immobility of the hind limbs. Dosing was suspended at the high dose level after the events in the first 4 animals. These events occurred at the end of the dosing interval therefore are not considered to correlate with C_{max} of NST-6179. However, continued dosing in the high dose level cohort was suspended and new dosing levels were evaluated the study.

Macroscopic examination of major organs and tissues in decedents did not reveal any changes that could be related to drug treatment or mis-dosing via gavage. A full histopathology report of decedents is pending but TK analysis indicates drug exposure at the well-tolerated intermediate dose levels is greater than the current stopping rules. At the high dose level, drug exposure is substantially above the current stopping rule area under the concentration-time curve (AUC) of 358,000 ng.h/mL from the 28-day monkey toxicity study. The Day 1 TK parameters in the mouse are presented in Table 2.

Table 2: Summary of the NST-6179 Toxicokinetic Parameters in the Mouse on Day 1 in the 13-week Oral (Gavage) Administration Toxicity Study (Labcorp Study Number 8423764)

Group	Dose (mg/kg)	Sex	AUC_{0-24} (ng.h/mL)
Low	70	Male	229,000
	70	Female	317,000
Intermediate	135	Male	400,000
	160	Female	587,000
High	200	Male	503,000
	250	Female	988,000

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose.

Based on these data, the dose levels were adjusted to account for the necessitated decrease in high dose group while maintaining an adequate dose separation between the 3 dosing cohorts. The adjusted dose levels for both males and females are 70 mg/kg/day, 120 mg/kg/day, and 170 mg/kg/day, with the adjustments made in the ongoing low and intermediate dose cohorts and a new cohort initiated at the high dose level.

The observed adverse events (AEs) in the mouse have been considered and, as TK data indicate AEs on Day 1 correlate with exposure (AUC) substantially above the current stopping rule, are not considered to be clinically relevant.

Section 1.7: Study Rationale

Previously read:

This is the first time NST-6179 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when NST-6179 is administered orally as single and multiple doses to healthy subjects. This information will help establish the doses and dosing regimen suitable for future studies in patients. ~~The study will also investigate the effects of food on the PK of NST-6179 prior to patient studies.~~

Now reads:

This is the first time NST-6179 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when NST-6179 is administered orally as single and multiple doses to healthy subjects. This information will help establish the doses and dosing regimen suitable for future studies in patients.

Section 1.8: Benefit-risk Assessment

Previously read:

Subjects in the current study will not receive any health benefit (beyond that of an assessment of their medical status) from participating in the study. The risks of participation are primarily those associated with adverse reactions to the study treatment, although there may also be some discomfort from collection of blood samples and other study procedures. More

information about the known and expected benefits, risks, and reasonably anticipated ~~adverse events (~~AEs~~)~~ associated with NST-6179 may be found in the Investigator's Brochure (IB).[1]

Now reads:

Subjects in the current study will not receive any health benefit (beyond that of an assessment of their medical status) from participating in the study. The risks of participation are primarily those associated with adverse reactions to the study treatment, although there may also be some discomfort from collection of blood samples and other study procedures.

The events observed in the toxicology studies to date are also considered in determining the overall benefit-risk of the Phase 1 study. All events occurred in these specific studies at systemic exposure exceeding the current established stopping rules. Based on the observed drug exposure, a significant safety margin exists for both the relevant species, as well as the mice in the carcinogenicity dose ranging study, at the proposed initial human dose of 50 mg. Therefore, the risk-benefit of conducting this first-in-human study remains unchanged and proposed stopping rules remain supported by data from the mouse, rat, and monkey.

More information about the known and expected benefits, risks, and reasonably anticipated AEs associated with NST-6179 may be found in the Investigator's Brochure (IB).[1]

Section 2.1: Objectives

Previously read:

The secondary objective~~s~~ of the study ~~are~~:

- to evaluate the single and multiple oral dose PK of NST-6179 in healthy male and female subjects
- ~~to determine the effect of food on the single oral dose PK of NST-6179 in healthy male and female subjects.~~

Now reads:

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

Section 2.2: Secondary Endpoints

Previously read:

The PK outcome endpoints of NST-6179 for Part A (single-ascending dose ~~and food effect [fed versus fasted dietary status at dosing]~~ in healthy subjects) are as follows:

Now reads:

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

Section 3.1.1: Part A

Previously read:

Part A will comprise a double-blind, single-ascending dose, sequential-group design ~~incorporating a food-effect evaluation~~. 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~~, except for Group A3, where each subject will participate in 2 treatment periods separated by a minimum of 7 days~~. Subjects will reside at the study site from Day -1 (the day before dosing) to Day 4 of each treatment period, as applicable. ~~It is the intent to conduct the assessment of the effect of food in Group A3; however, based on emerging data, this may be conducted in a different dose group in Part A.~~

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~~, A2, and A4~~ to A6
It is planned for each subject in Groups A1~~, A2, and A4~~ 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.

~~**Group A3**~~
~~It is planned for each subject in Group A3 to have the same treatment in both treatment periods, such that each subject will receive 2 single doses of NST-6179 or placebo during the study. On Day 1 in Treatment Period 1, doses will be administered in the fasted state in accordance with a randomisation schedule. On Day 1 in Treatment Period 2, doses will be given 30 minutes after starting a standard high-fat breakfast. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change based on emerging data.~~

Additional Groups (Groups A7 to A9)
If it is decided to enrol additional groups (Section 3.3)~~, the effect of food on the PK of NST-6179 may further be evaluated as described for Group A3. However,~~ 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. ~~For groups participating in a food-effect evaluation, sentinel dosing will only be utilised in Treatment Period 1 when NST-6179 or placebo are administered in the fasted state.~~

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 Figure 2~~ and the planned dose levels are presented in Figure 3. A Schedule of Assessments is presented in Appendix 6.

Now reads:

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 (Section 3.3) 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. A Schedule of Assessments is presented in Appendix 6.

Section 3.1.1: Part A

Previously read:

Figure: 1: Study Schematic (Part A) for Groups A1~~, A2, and A4~~ to A6

Now reads:

Figure 1: Study Schematic (Part A) for Groups A1 to A6

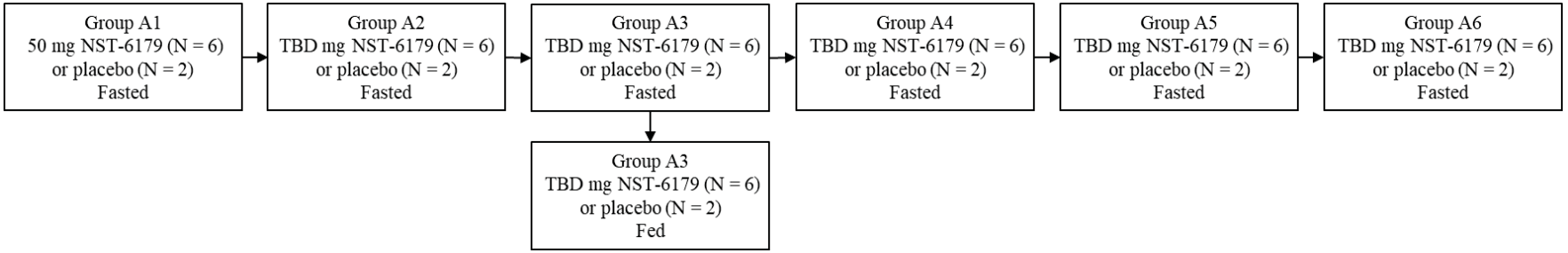
Section 3.1.1: Part A

Figure 2 removed.

Section 3.1.1: Part A

Previously read:

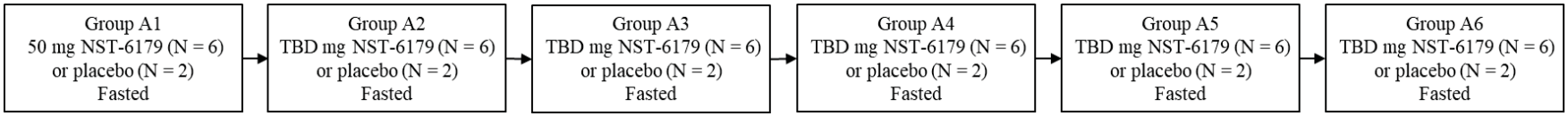
Figure ~~3~~: Planned Dose Levels for Part A



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.
~~Although the food-effect evaluation is planned to occur in Group A3, this may be subject to change based on emerging data and may be conducted in a different dose group in Part A.~~

Now reads:

Figure **2**: Planned Dose Levels for 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.

Section 3.1.1: Part A

Previously read:

The total planned duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 6 weeks ~~for subjects not participating in the food-effect evaluation, and approximately 8 weeks for those participating in the food-effect evaluation~~.

Now reads:

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

Section 3.1.2: Part B

Previously read:

In each of Groups B1 to B4, 8 subjects will receive NST-6179 and 2 subjects will receive placebo. ~~The dietary state for dosing in Part B will be subject to review of the PK data from the fed/fasted comparison in Part A or earlier groups in Part B.~~ For all subjects, dosing is planned to be QD on Days 1 to 14, inclusive. However, ~~the dietary state (including fasting requirements and meal compositions),~~ dosing frequency~~,~~ and ~~dosing~~ duration in Part B may be changed following review of data from groups in Part A or earlier groups in Part B (Section 3.4). The total daily dose administered will not be predicted to exceed an exposure shown to be safe and well tolerated in Part A. 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 (Section 3.4). There will be a minimum of 14 days between dose escalations for each group in Part B.

Now reads:

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 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 (Section 3.4). The total daily dose administered will not be predicted to exceed an exposure shown to be safe and well tolerated in Part A. 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 (Section 3.4). There will be a minimum of 14 days between dose escalations for each group in Part B.

Section 3.3: Additional Groups

Previously read:

Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A, and up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B. ~~This may include additional groups in Part A to explore the effect of food (ie, additional 2-period groups).~~ There will be no further dose escalation in these additional groups if dose escalation stopping criteria have been met and a dose level cannot be repeated if it previously met a stopping criterion. The requirement for additional groups will be agreed with the sponsor and documented in the trial master file (TMF).

Now reads:

Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A, and up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B. There will be no further dose escalation in these additional groups if dose escalation stopping criteria have been met and a dose level cannot be repeated if it previously met a stopping criterion. The requirement for additional groups will be agreed with the sponsor and documented in the trial master file (TMF).

Section 3.4: Discussion of Study Design, Including the Choice of Control Groups

Previously read:

For Part A and Part B of the study, a sequential-group, ascending-dose design has been chosen for safety reasons as NST-6179 is in the early stages of clinical development, with Part A of the study being the first time it will be administered to humans. Oral doses have been chosen for both parts of the study, as this is the intended clinical route of administration. ~~A 2-period design has been chosen for the food-effect arm, as this gives a within-subject assessment of the influence of food on the PK of NST-6179 and so increases the power of the study for the given number of subjects.~~

Now reads:

For Part A and Part B of the study, a sequential-group, ascending-dose design has been chosen for safety reasons as NST-6179 is in the early stages of clinical development, with Part A of the study being the first time it will be administered to humans. Oral doses have been chosen for both parts of the study, as this is the intended clinical route of administration.

Section 3.4: Discussion of Study Design, Including the Choice of Control Groups

Previously read:

Based upon the non-clinical data, the duration of each treatment period is considered adequate to achieve the study objectives. ~~Where applicable, an interval of at least 7 days between treatment periods in the food-effect arm is considered adequate to prevent carryover of NST-6179.~~

Now reads:

Based upon the non-clinical data, the duration of each treatment period is considered adequate to achieve the study objectives.

Section 3.5: Selection of Doses in the Study

Previously read:

Table 4: Proposed Investigational Medicinal Product Dose Levels for Parts A and B

Study Part	Group	Subject Numbers	Treatment Period 1	~~Treatment Period 2~~
Part A	A1	0101 – 0108	50 mg or placebo	~~NA~~
	A2	0109 – 0116	TBD mg or placebo	~~NA~~
	A3[a]	0117 – 0124	TBD mg or placebo~~(fasted)~~	~~TBD mg or placebo (fed)~~
	A4	0125 – 0132	TBD mg or placebo	~~NA~~
	A5	0133 – 0140	TBD mg or placebo	~~NA~~
	A6	0141 – 0148	TBD mg or placebo	~~NA~~
	A7[b]	0149 – 0156	TBD	~~TBD~~
	A8[b]	0157 – 0164	TBD	~~TBD~~
	A9[b]	0165 – 0172	TBD	~~TBD~~
Part B	B1	0201 – 0210	TBD mg or placebo	~~NA~~
	B2	0211 – 0220	TBD mg or placebo	~~NA~~
	B3	0221 – 0230	TBD mg or placebo	~~NA~~
	B4	0231 – 0240	TBD mg or placebo	~~NA~~
	B5[e]	0241 – 0250	TBD	~~NA~~
	B6[e]	0251 – 0260	TBD	~~NA~~
	B7[e]	0261 – 0270	TBD	~~NA~~

Abbreviations: NA = not applicable; PK = pharmacokinetic(s); TBD =to be determined.

~~a. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change based on emerging data and may be conducted in a different dose group in Part A.~~

~~b.~~ Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A. ~~This may include additional groups in Part A to explore the effect of food (ie, additional 2-period groups).~~

~~e.~~ Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B.

Now reads:

Table **6**: Proposed Investigational Medicinal Product Dose Levels for Parts A and B

Study Part	Group	Subject Numbers	Treatment Period 1
Part A	A1	0101 – 0108	50 mg or placebo
	A2	0109 – 0116	TBD mg or placebo
	A3	0117 – 0124	TBD mg or placebo
	A4	0125 – 0132	TBD mg or placebo
	A5	0133 – 0140	TBD mg or placebo
	A6	0141 – 0148	TBD mg or placebo
	A7[a]	0149 – 0156	TBD
	A8[a]	0157 – 0164	TBD
	A9[a]	0165 – 0172	TBD
Part B	B1	0201 – 0210	TBD mg or placebo
	B2	0211 – 0220	TBD mg or placebo
	B3	0221 – 0230	TBD mg or placebo
	B4	0231 – 0240	TBD mg or placebo
	B5[b]	0241 – 0250	TBD
	B6[b]	0251 – 0260	TBD
	B7[b]	0261 – 0270	TBD

Abbreviations: NA = not applicable; PK = pharmacokinetic(s); TBD =to be determined.

a. Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in Part A.

b. Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 10 subjects (8 active:2 placebo) may be included in Part B.

Section 4.2.1: Part A and B

Exclusion criterion #19 removed.

Section 5.2: Study Treatment Administration

Previously read:

Each dose of NST-6179 and placebo will be administered orally with approximately 240 mL of room temperature water. In Part A, all doses will be administered after an overnight fast of at least 10 hours~~, with the exception of Group A3, where the dose given in Treatment Period 2 will be administered 30 minutes after starting a high-fat breakfast~~. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing. ~~The dietary status for dosing in Part B will be determined following review of the PK data from the fed/fasted comparison in Part A or earlier groups in Part B.~~

Now reads:

Each dose of NST-6179 and placebo will be administered orally with approximately 240 mL of room temperature water. In Part A **and on the days with intensive PK assessments in Part B**, all doses will be administered after an overnight fast of at least 10 hours. With the

exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing.

Section 5.3: Randomisation

Previously read:

In Part A, 6 subjects per group will be randomly assigned to receive NST-6179 and 2 subjects per group will be randomly assigned to receive placebo. ~~Subjects participating in a food-effect evaluation will receive the same treatment in Treatment Periods 1 and 2.~~ For all groups in Part A, sentinel dosing will occur whereby 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining 6 subjects (5 active and 1 placebo) will be dosed after 24 hours. ~~For groups participating in a food-effect evaluation, sentinel dosing will only be utilised in Treatment Period 1 when NST-6179 or placebo are administered in the fasted state.~~

Now reads:

In Part A, 6 subjects per group will be randomly assigned to receive NST-6179 and 2 subjects per group will be randomly assigned to receive placebo. For all groups in Part A, sentinel dosing will occur whereby 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining 6 subjects (5 active and 1 placebo) will be dosed after 24 hours.

Section 6.2: Diet

Previously read:

On the days with intensive PK assessments ~~(Day 1 for Part A and Days 1 and 14 for Part B)~~, meals will be identical for each group ~~with the exception of the high-fat breakfast for Group A3 (or any other group participating in a food-effect evaluation)~~.

~~**Fasted doses:**~~ On Day 1 in Part A, subjects will be fasted for at least 10 hours prior to dosing until approximately 4 hours after dosing, when lunch will be provided (lunch times on Day 1 will be staggered between subjects to ensure this). With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing. Meals will be provided as appropriate at other times. Other than the fluid restrictions on dosing days, water will be freely available at all times.

~~**Fed doses:** Subjects in Group A3 (or any other group participating in a food-effect evaluation) in Treatment Period 2 will consume a high-fat breakfast (contents are detailed in) before dosing. Subjects should start the meal 30 minutes prior to administration of the IMP. Study subjects should eat this meal in 30 minutes or less. The drug product should be administered 30 minutes after the start of the meal. Subjects will be fasted for approximately 4.5 hours after dosing. With the exception of milk given with the high-fat breakfast and water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing~~

~~**Table 5: High-fat Breakfast Content**~~

~~**High-fat Breakfast**~~
~~120 g fried eggs (2 eggs) in vegetable oil~~
~~50 g bacon (2 rashers)~~
~~72 g toasted white bread (2 slices)~~
~~13 g butter (2 pats)~~
~~108 g hash brown (3 each)~~
~~240 g whole milk~~
~~Total calories: 973 kcal~~

~~This high-fat meal contains the equivalent of approximately 150 protein calories, 250 carbohydrate calories, and 500 to 600 fat calories.~~

In Part B, ~~the time interval between meals and dosing will be determined by the PK data from Part A and will be documented in the TMF~~. Meals will be provided as appropriate at other times. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to dosing until 2 hours after dosing. Other than these fluid restrictions, water will be freely available at all times.

Now reads:

On the days with intensive PK assessments, meals will be identical for each group.

On Day 1 in Part A, subjects will be fasted for at least 10 hours prior to dosing until approximately 4 hours after dosing, when lunch will be provided (lunch times on Day 1 will be staggered between subjects to ensure this). With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing. Meals will be provided as appropriate at other times. Other than the fluid restrictions on dosing days, water will be freely available at all times.

In Part B, **on days with intensive PK assessments, subjects will be fasted for at least 10 hours prior to dosing until approximately 4 hours after dosing**. Meals will be provided as appropriate at other times. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to dosing until 2 hours after dosing. Other than these fluid restrictions, water will be freely available at all times.

Section 8.3: Pharmacokinetic Analyses

Previously read:

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-tlast}}$, $AUC_{0\text{-}\infty}$, and C_{max} on Day 1 in Part A and $AUC_{0\text{-}\tau}$ and C_{max} on Day 14 in Part B. The PK parameters will be analysed using a power model. However, if the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be natural log-transformed and analysed using an analysis of variance model.

~~A statistical analysis will be conducted to investigate the food effect on the treatment for $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, C_{max}, and t_{max} in Part A (food-evaluation group only). The natural log-transformed $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} parameters will be analysed using a mixed model. The model will include actual treatment as fixed effect and subject as a random effect, where treatment includes the fasting status. The t_{max} parameter will be analysed using the Wilcoxon signed-rank test.~~

Now reads:

A statistical analysis will be conducted to investigate the dose proportionality of $AUC_{0\text{-}tlast}$, $AUC_{0\text{-}\infty}$, and C_{max} on Day 1 in Part A and $AUC_{0\text{-}\tau}$ and C_{max} on Day 14 in Part B. The PK parameters will be analysed using a power model. However, if the assumption of linearity is ruled unacceptable for any PK parameter, its corresponding PK parameter normalised by dose administered will be natural log-transformed and analysed using an analysis of variance model.

Appendix 3: Total Blood Volume

Previously read:

Part A

The following blood volumes will be withdrawn for each subject ~~not participating in a food-effect evaluation~~:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	5	37.5
Serology	3.5	1	3.5
Coagulation	1.8	5	9.0
NST-6179 pharmacokinetics[b]	2.0	19	38.0
Total:			88.0

Abbreviation: FSH = follicle-stimulating hormone.
a. Includes pregnancy and FSH tests.
b. Includes 3 additional samples that may be taken.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 140 mL.

~~The following blood volumes will be withdrawn for each subject participating in a food-effect evaluation:~~

	~~Volume per blood sample (mL)~~	~~Maximum number of blood samples~~	~~Total amount of blood (mL)~~
~~Clinical laboratory evaluations[a]~~	~~7.5~~	~~8~~	~~60.0~~
~~Serology~~	~~3.5~~	~~1~~	~~3.5~~
~~Coagulation~~	~~1.8~~	~~8~~	~~14.4~~
~~NST-6179 pharmacokinetics[b]~~	~~2.0~~	~~37~~	~~74.0~~
~~Total:~~			~~151.9~~

~~Abbreviation: FSH = follicle-stimulating hormone.~~
~~a. Includes pregnancy and FSH tests.~~
~~b. Includes 3 additional samples (per treatment period) that may be taken.~~

~~If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 205 mL.~~

Now reads:

Part A

The following blood volumes will be withdrawn for each subject:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	5	37.5
Serology	3.5	1	3.5
Coagulation	1.8	5	9.0
NST-6179 pharmacokinetics[b]	2.0	19	38.0
Total:			88.0

Abbreviation: FSH = follicle-stimulating hormone.
a. Includes pregnancy and FSH tests.
b. Includes 3 additional samples that may be taken.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 140 mL.

Appendix 6: Schedule of Assessments

Reference to food-effect removed, and footnotes edited, as applicable.

Appendix 6: Schedule of Assessments

Previously read:

Schedule of Assessments – Part A (Single Dose in Healthy Subjects)

Study Procedures	**Screening (Day -28 to Day -2)**	**Treatment Period 1 ~~(and 2 for Group A3)~~[a]**		**Follow-up (10 to 14 days postdose)**
		Day -1	**Days 1 to 4**	
NST-6179 or placebo			Day 1 (0 hour)~~ (30 minutes after starting a high-fat breakfast in Treatment Period 2 for Group A3[a])~~	
Safety and tolerability:				
Clinical chemistry, haematology, and urinalysis	X	X	Predose and ~~72~~ hours postdose	X
Coagulation	X	X	Predose and ~~72~~ hours postdose	X

Now reads:

Schedule of Assessments – Part A (Single Dose in Healthy Subjects)

Study Procedures	**Screening (Day -28 to Day -2)**	**Treatment Period 1**		**Follow-up (10 to 14 days postdose)**
		Day -1	**Days 1 to 4**	
NST-6179 or placebo			Day 1 (0 hour)	
Safety and tolerability:				
Clinical chemistry, haematology, and urinalysis	X	X	Predose and **48** hours postdose	X
Coagulation	X	X	Predose and **48** hours postdose	X

Version 2 to Version 3

NST-6179 is a novel, orally administered, fully-synthetic medium chain fatty acid (MCFA) analogue which is being developed for the treatment or the treatment of Intestinal failure-associated liver disease (IFALD), and other potential indications. Study NST-6179-01 is a Phase 1, first-in-human, randomised, placebo-controlled single- and multiple-ascending dose study to evaluate the safety, tolerability, and pharmacokinetics (PK) of NST-6179 (EudraCT Number: 2021-000839-31). The study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) on 28 May 2021 and by the Ethics Committee (EC) on 28 May 2021. The completed non-clinical program primary pharmacology studies in relevant *in vivo* mouse models, safety pharmacology studies *in vitro* and *in vivo* in the rat and monkey, *in vitro* mouse, rat, rabbit, dog, monkey and human plasma protein binding, repeated dose (28 day) toxicity study in the rat and monkey, and genotoxicity assessment. Further details of these studies are included in the Investigator Brochure (Version 1.0, 09 March 2021), all of which supported the initiation of the Phase 1, first-in-human clinical study.

Subsequently, a 13-week repeat dose oral toxicity study in the monkey was initiated on 16 June 2021 and is currently ongoing. Single daily oral doses of 0, 60, 210, or 350 mg/kg/day have been administered in both male and female monkeys and generally well tolerated to date. Mild spurious vomiting was observed at 350 mg/kg/day in both males and females, which declined in frequency over time. Additionally, an 8% decrease in bodyweight was also observed during the first week of dosing in both male and female animals at the high dose as well, which subsequently increased back to baseline at Day 7. No other relevant clinical findings have been observed to date at any dose level in either male or female monkeys. However, on Day 16, a single female monkey in the high dose group was found dead following dosing on Day 15. The decedent female appeared to be in general good health and displayed no clinical signs up through and postdose on Day 15. Dosing was temporarily held in the high dose female cohort and, out of caution, was restarted at a lower dose of 300 mg/kg/day, as well as increasing the frequency of postdose monitoring. An investigation was initiated immediately which include an expedited macroscopic post-mortem examination and clinical pathology assessment as well as a review of all study data prior to the event for the decedent monkey and the other animals in the study. The Sponsor placed a temporary hold on the Phase 1 clinical study on 02 July 2021 with notification to the MHRA and EC in accordance with the required timelines.

A macroscopic post-mortem examination was performed on the decedent female and all major organs and tissues were processed for histopathological examination. The absence of advanced autolysis at macroscopic post-mortem indicated the female died relatively soon prior to the post-mortem examination. Although an exact time of death cannot be established, the relative timeframe of death was close to 24 hours after the Day 15 dose, when minimal drug concentrations levels would have been present. Microscopically, minimal alveolar/interstitial inflammation was noted which is consistent with post-aspiration effects. However, the minimal severity and distribution is considered not to be the cause of mortality. The majority of tissues were well preserved but some autolysis was noted in the gall bladder and gastrointestinal tract and to a lesser extent liver, pancreas, and salivary glands. Although histopathological assessment has not identified the cause of death, there was no evidence of drug-related changes or injury.

Further detailed examination of the clinical condition and study data from the decedent animal was also reviewed to determine any underlying condition existed as a potential cause of death. Clinical chemistry and urinalysis parameters were all normal. Haematology parameters were all within normal limits, with the exception of minimal increases in white cells and absolute neutrophil count. However, no evidence of infection was noted associated with these elevations. A minimal decrease in red cells, haemoglobin and packed cell volume was noted. None of these changes were considered to be clinically meaningful nor contributory to the cause of death. Assessment of the decedents ECG indicated minor anomalies considered not clinically relevant. All of the clinical and laboratory data preceding the event, as well as the post-mortem assessments, have been peer reviewed by two pathologists, both of whom concur with the observations and the lack of evidence of a drug-related event.

A review of the data from the prior 28-day repeat dose oral toxicity study in monkeys established 350 mg/kg/day was the no-observed-adverse-effect level (NOAEL) level (Covance: 8423746). No clinical signs and symptoms or mortality was observed at this dose level. Importantly, in the ongoing 13-week study, NST-6179 continues to be well tolerated in all other males and females at all dosages.

In summary, the investigation will continue to attempt to establish a cause of death but as of this date there is no clear evidence that this was a drug-related event. However, in accordance with the temporary hold, all patient-related activities were stopped and no participant has been randomized or dosed with NST-6179 at this time. Additionally, the Sponsor is proposing the following changes to Study NST-6179-01 as a precaution in response to the observed event:

1. Revision to Dose Escalation Stopping Criterion based on PK.
   The PK stopping rule in Protocol Version 2 (26 April 2021) was based on the female maximum observed concentration (C_{max}) and area under the concentration-time curve from time zero to 24 hours postdose (AUC_{0-24}) at the NOAEL in the cynomolgus monkey 28-day repeat dose oral toxicity study at the high dose level of 350 mg/kg/day, with values of 59,100 ng/mL for C_{max} and 440,000 ng.h/mL for AUC_{0-24}. Although there is no clear relationship between drug treatment and mortality of the female monkey in the current study, we propose a revision to ensure the participant safety. The proposed revision incorporates the AUC_{0-24} in the female cynomolgus monkey at the intermediate dose level (175 mg/kg/day) in the 28-day oral toxicity study (AUC_{0-24} of 358000 ng.hr/mL); a decrease from the current AUC_{0-24}. In the 28-day oral monkey toxicity study, the C_{max} in the female high dose 350 mg/kg/day (59,100 ng/mL) is lower than that at the low and intermediate dose levels of 60 mg/kg (C_{max} of 72,400 ng/mL) and 175 mg/kg (C_{max} of 147,000 ng/mL) respectively. Additionally, no adverse observations have been recorded that correlate with C_{max}, including the female mortality in the 13-week study. Therefore, no change to the C_{max} stopping rule is proposed at this time.
1. Decrease of Initial Proposed Human Starting Dose.
   The initial proposed human starting dose of 100 mg is well within the safety margins with projected exposures significantly lower than the proposed stopping

rules. However, we are proposing to decrease the starting dose to 50 mg to further mitigate any risk to study subjects.

2. Removal of Part C.
   The inclusion of Part C (open-label, single oral dose study to evaluate the PK of NST-6179 in patients receiving home parenteral nutrition, for short bowel syndrome, but with a functional duodenum, was planned to be incorporated in a future substantial amendment). The initial study plan was to evaluate the safety, tolerability and PK of NST-6179 exposure in healthy subjects followed by an evaluation of relevant doses in the intended population. However, NorthSea Therapeutics believe it is prudent to evaluate additional 13-week toxicology data in the context of the healthy participant data prior to dosing in a patient population. Therefore, we are removing the patient cohort from the current single-ascending dose/multiple-ascending dose study.
3. Update of Sponsor Signatory and Sponsor Project manager due to personnel changes.
4. As of June 2021, there has been a name change with Covance being rebranded as Labcorp.

Minor changes:

5. The synopsis was updated according to changes in the protocol body, as applicable.
6. The amendment/version number and date were updated throughout the protocol.
7. The header was updated to reflect Covance being rebranded as Labcorp.
8. Abbreviations were added and/or removed, as appropriate.

A detailed summary of changes is presented below:

For each section, deleted text is shown in ~~strikethrough~~, and new text is shown in **bold**.

Title

Previously read:

A Phase 1, First Time in Human Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of NST-6179 in Healthy Subjects~~ and Patients Requiring Home Parenteral Nutrition (HPN) for Short Bowel Syndrome~~

Now reads:

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

Title Page

Previously read:

~~Covance~~ Study Number: 8462139

Now reads:

Labcorp Drug Development Study Number: 8462139

Title Page

Previously read:

Study Site:
~~Covance~~ Clinical Research Unit (CRU) Ltd.

Now reads:

Study Site:
Labcorp Clinical Research Unit (CRU) Ltd.

Title Page

Previously read:

Sponsor Signator~~y~~:
Dr ~~Patrick Round, MBBS, FFPM~~

Now reads:

Sponsor Signator**ies**:
Dr **Stephen Rossi, PharmD**
Dr Stephen Harrison, MD

SPONSOR APPROVAL

Previously read:

Sponsor Signatory:
Dr ~~Patrick Round, MBBS, FFPM~~
~~Medical Director~~
NorthSea Therapeutics B.V.

Now reads:

Sponsor Signatory:
Dr **Stephen Rossi, PharmD**
Chief Development Officer
NorthSea Therapeutics B.V.

Dr Stephen Harrison, MD
Chief Medical Officer
NorthSea Therapeutics B.V.

STUDY IDENTIFICATION

Previously read:

Sponsor's Study Contact	~~Lis Mable~~ ~~Project~~ Manager NorthSea Therapeutics B.V. Paasheuvelweg 25-C6 1105 BP, Amsterdam The Netherlands
Sponsor's Medical Contact	Dr ~~Patrick Round, MBBS, FFPM~~ Medical ~~Director~~ NorthSea Therapeutics B.V. Paasheuvelweg 25 C6 1105 BP, Amsterdam The Netherlands Tel (after hours): +44 (0)7979 502 770
Study Site	~~Covance~~ Clinical Research Unit (CRU) Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom
Principal Investigator	Dr Jim Bush, MBChB, PhD, MRCS, FFPM, GFMD VP CPS Medical Services ~~Covance~~ CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0) 113 301 3644 Tel (after hours): +44 (0) 113 301 3500
Project Physician	Dr Samuel Israel, MB:BS, MRCOG, FRCOG Clinical Research Physician ~~Covance~~ CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0) 113 301 3527
Clinical Laboratory	~~Covance Clinical Pathology Services~~ Otley Road Harrogate, HG3 1PY United Kingdom
Statistician	Izabela Antys, BSc MSc GradStat ~~Covance Biometrics~~

Now reads:

Sponsor's Study Contact	**Kathline Kim** **Senior Clinical Trial** Manager NorthSea Therapeutics B.V. Paasheuvelweg 25-C6 1105 BP, Amsterdam The Netherlands **Tel: +1 510 282 3024** **Email: kathline.kim@northseatherapeutics.com**
Study Director	**Dr Stephen Rossi, PharmD** **Chief Development Officer** **NorthSea Therapeutics B.V.** **Paasheuvelweg 25-C6** **1105 BP, Amsterdam** **The Netherlands** **Tel: +1 415 572 0596** **Email: stephen.rossi@northseatherapeutics.com**
Sponsor's Medical Contact	Dr **Stephen Harrison, MD** **Chief** Medical **Officer** NorthSea Therapeutics B.V. Paasheuvelweg 25-C6 1105 BP, Amsterdam The Netherlands Tel: **+1 210 288 5868** **Email: stephen.harrison@northseatherapeutics.com**
Study Site	**Labcorp** Clinical Research Unit (CRU) Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom
Principal Investigator	Dr Jim Bush, MBChB, PhD, MRCS, FFPM, GFMD VP CPS Medical Services **Labcorp** CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0) 113 301 3644 Tel (after hours): +44 (0) 113 301 3500
Project Physician	Dr Samuel Israel, MB:BS, MRCOG, FRCOG Clinical Research Physician **Labcorp** CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0) 113 301 3527

Clinical Laboratory	**Labcorp Early Development Laboratories Ltd.** Otley Road Harrogate, HG3 1PY United Kingdom
Statistician	Izabela Antys, BSc MSc GradStat **Labcorp Drug Development**

Section 1.3: Summary of Safety Pharmacology

Previous read:

Four safety pharmacology studies were ~~conducted~~ (all ~~were~~ conducted to Good Laboratory Practice [GLP]).

Now reads:

Four safety pharmacology studies were **completed** (all conducted to Good Laboratory Practice [GLP]).

Section 1.4.3 (new section): 13-week Oral (Gavage) Toxicity and Toxicokinetic Study in the Monkey with a 2-week Recovery Period

This is an ongoing study objective to evaluate the toxicity and determine the toxicokinetics (TK) of NST-6179 when administered daily via oral gavage to the monkey for at least 13 weeks and to assess the reversibility or persistence of any effects after a 4-week recovery phase following doses of 60 mg/kg/day (low dose), 210 mg/kg/day (intermediate dose), and 350 mg/kg/day (high dose). The purpose of the study is to establish dose levels for future long term toxicity studies. A high dose level of 350 mg/kg/day was selected based on results from a 28-day repeat dose toxicity study (Covance 8423746). An original high dose of 500 mg/kg/day was not tolerated, resulting in two early sacrifices and postdose observations of decreased activity, ataxia, semi-closed eyes, hunched posture, and vomiting. Following a 30-day washout period, a revised high dose level of 350 mg/kg/day was investigated over 28 days, which was tolerated with no mortality and transient mild and occasional postdose observations of decreased activity, hunched posture, and vomiting that diminished throughout the 28 days dosing. An intermediate dose level of 210 mg/kg/day was selected as an alternative high dose level for future studies if the selected high dose level was not tolerated. A low dose level of 60 mg/kg/day was expected to be tolerated in-life, with no postdose observations or body weight effect noted following 28 days repeat administration at this dose level during the previous toxicity study. The cynomolgus monkey was selected as the relevant species because of the similarity of monkeys to humans in species specific cross-reactivity of test article. An *in vitro* metabolism study (Covance study 8423743) indicated the cynomolgus monkey is the preferred choice of large animal species due to the presence of specific human metabolites, which are not present in other species.

Animals were dosed through Day 15 and NST-6179 was generally well tolerated with only mild spurious vomiting and an 8% decrease in bodyweight observed in the early stages, both of which returned to baseline status over time. No other relevant clinical

findings have been observed to date at any dose level in either male or female monkeys. However, on Day 16, a single female monkey in the high dose group was found dead following dosing on Day 15. The decedent female appeared to be in general good health and displayed no clinical signs up through and postdose on Day 15. Dosing was temporarily held in the high dose female cohort and out of caution was restarted at a lower dose of 300 mg/kg/day and the frequency of postdose monitoring was increased. A macroscopic post-mortem examination showed no advanced autolysis at macroscopic post-mortem indicated the female died relatively soon prior to the post-mortem examination. Although an exact time of death cannot be established, the relative time frame of death was close to 24 hours after the Day 15 dose, when minimal drug concentrations levels would have been present. Microscopically, minimal alveolar/interstitial inflammation was noted which is consistent with post aspiration effects. However, the minimal severity and distribution is considered not to be the cause of mortality. The majority of tissues were well preserved but some autolysis was noted in the gall bladder and gastrointestinal tract and to a lesser extent liver, pancreas and salivary glands. Further detailed examination of the clinical condition and study data from the decedent animal was also reviewed to determine any underlying condition existed as a potential cause of death. Clinical chemistry and urinalysis parameters were all normal. Haematology parameters were all within normal limits, with the exception of minimal increases in white cells and absolute neutrophil count. However, no evidence of infection was noted associated with these elevations. A minimal decrease in red cells, haemoglobin and packed cell volume was noted. None of these changes were felt to be clinically meaningful nor contributory to the cause of death. Assessment of the decedents ECG indicated minor anomalies considered not clinically relevant.

A review of the data from the prior 28-day repeat dose oral toxicity study in monkeys established 350 mg/kg/day was the NOAEL level (Covance 8423746). No clinical signs and symptoms or mortality was observed at this dose level. Importantly, in the ongoing 13-week study, NST-6179 continues to be well tolerated in all other males and females at all dosages up to 28 days of dosing. In summary, all of the clinical and laboratory data preceding the event, as well as the post-mortem assessments, have been peer reviewed by two pathologists, both of whom concur with the observations and the lack of evidence of a drug-related event.

Section 1.7: Study Rationale

Previously read:

This is the first time NST-6179 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when NST-6179 is administered orally as single and multiple doses to healthy subjects. ~~In addition, this study aims to evaluate the PK of a single dose of NST-6179 in patients receiving home parenteral nutrition (HPN) for short bowel syndrome.~~ This information~~, together with the PK data from healthy subjects,~~ will help establish the doses and dosing regimen suitable for future studies in patients. The study will also investigate the effects of food on the PK of NST-6179 prior to patient studies.

Now reads:

This is the first time NST-6179 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when NST-6179 is administered orally as single and multiple doses to healthy subjects. This information will help establish the doses and dosing regimen suitable for future studies in patients. The study will also investigate the effects of food on the PK of NST-6179 prior to patient studies.

Section 2.1: Objectives

Previously read:

The primary objectives of the study are:

- to assess the safety and tolerability of single and multiple oral doses of NST-6179 in healthy male and female subjects
- ~~to assess the safety and tolerability of single doses of NST-6179 in male and female patients receiving HPN for short bowel syndrome, but with a functional duodenum.~~

The secondary objectives of the study are:

- to evaluate the single and multiple oral dose PK of NST-6179 in healthy male and female subjects
- to determine the effect of food on the single oral dose PK of NST-6179 in healthy male and female subjects
- ~~to evaluate the PK profile in male and female patients receiving HPN for short bowel syndrome, but with a functional duodenum.~~

Now reads:

The primary objectives of the study are:

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

The secondary objectives of the study are:

- to evaluate the single and multiple oral dose PK of NST-6179 in healthy male and female subjects
- to determine the effect of food on the single oral dose PK of NST-6179 in healthy male and female subjects**.**

Section 3: Investigational Plan

Previously read:

This will be a partly double-blind, randomised, placebo-controlled, single and multiple oral dose study conducted in 3 parts. Part A and Part B will be double-blind, randomised,

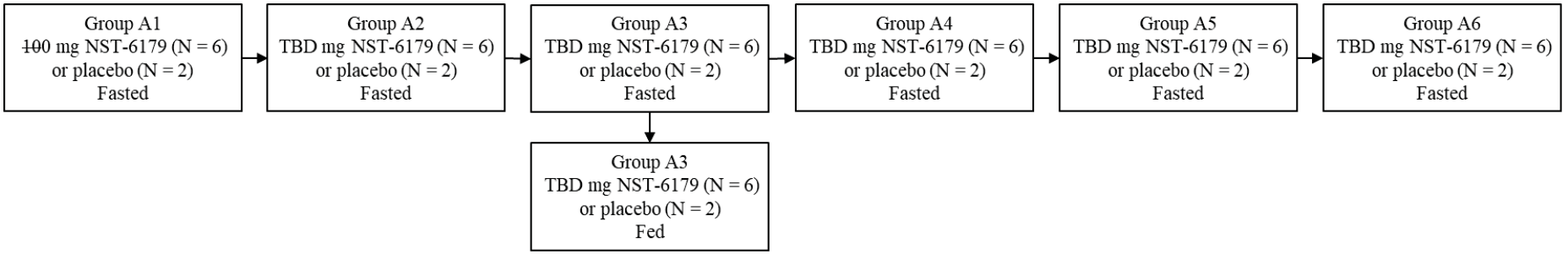
placebo-controlled, with subjects receiving single (Part A) and multiple (Part B) oral doses. ~~Part C will be open-label, non-randomised with patients receiving a single oral dose.~~

Now reads:

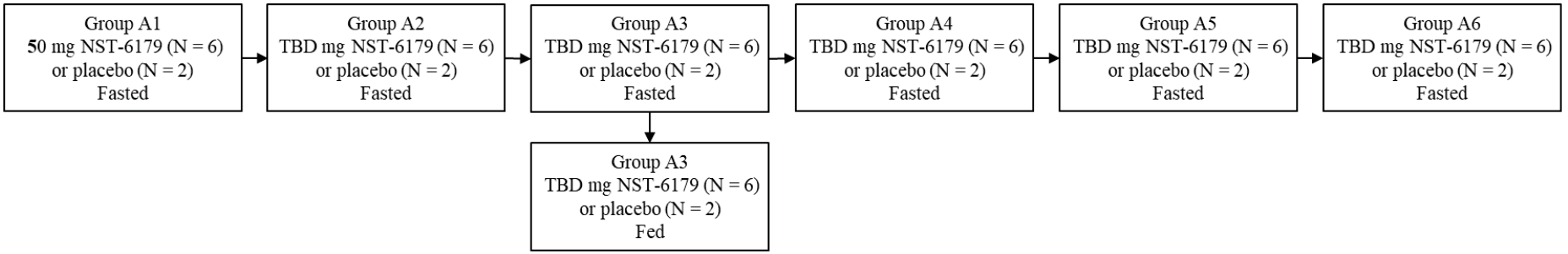
This will be a partly double-blind, randomised, placebo-controlled, single and multiple oral dose study conducted in 3 parts. Part A and Part B will be double-blind, randomised, placebo-controlled, with subjects receiving single (Part A) and multiple (Part B) oral doses.

Figure 3: Planned Dose Levels for Part A

Previously read:



Now reads:



Section 3.1.3: Part C

~~Part C will be an open-label, single oral dose study to evaluate the PK of NST-6179 in patients receiving HPN for short bowel syndrome, but with a functional duodenum. Four to 6 patients will be studied in a single group (Group C1).~~

~~Part C will be described in a future substantial protocol amendment.~~

Section 3.5: Selection of Doses in the Study

Previously read:

Compared to the HED for the NOAEL in the rat, the most sensitive species (ie, that with the lowest HED), a starting dose of ~~100~~ mg in Part A will correspond to a dose of:

$$\frac{\text{~~10~~0 mg}}{\text{60 kg}} = \text{~~1.67~~ mg/kg in a 60-kg subject}$$

and a safety margin of:

$$\frac{\text{72 mg/kg}}{\text{~~1.67~~ mg/kg}} = \textbf{~~43~~-fold}$$

The approximate effective dose of NST-6179 in an efficacy model in the mouse was 70 mg/kg (Section 1.5.3), which corresponds to a HED of:

Mouse: 70 mg/kg × 0.08 = **5.6 mg/kg**
(equivalent to approximately 336 mg in a 60-kg subject)

Where 0.08 is the conversion factor to extrapolate the animal dose to the HED based on body surface area.[2] Compared to the HED for the approximate effective dose in the mouse (ie, dose shown to have pharmacological activity in the mouse model), a starting dose of ~~10~~0 mg in Part A will be approximately ~~3~~-fold lower than a dose expected to have pharmacological activity:

$$\frac{\text{5.6 mg/kg}}{\text{~~1.67~~ mg/kg}} = \textbf{~~3.4~~-fold}$$

Evaluation of protein binding indicates the unbound drug fraction is 4% in mouse compared to 1.78% in human. The proposed starting dose based on unbound drug is therefore approximately ~~9~~ times lower than the effective dose. Although there is no empirical data that defines a pharmacologically inactive dose in the mouse it is considered unlikely that a human starting dose of ~~10~~0 mg would elicit significant pharmacological activity, if any. Furthermore metabolism in vitro is notably lower in mouse compared to human. Exposure to parent drug in human is likely to be proportionately less than mouse based on equivalent doses.

Now reads:

Compared to the HED for the NOAEL in the rat, the most sensitive species (ie, that with the lowest HED), a starting dose of **50** mg in Part A will correspond to a dose of:

$$\frac{\mathbf{5}0 \text{ mg}}{60 \text{ kg}} = \mathbf{0.83} \text{ mg/kg in a 60-kg subject}$$

and a safety margin of:

$$\frac{72 \text{ mg/kg}}{\mathbf{0.83} \text{ mg/kg}} = \mathbf{87\text{-fold}}$$

The approximate effective dose of NST-6179 in an efficacy model in the mouse was 70 mg/kg (Section 1.5.3), which corresponds to a HED of:

Mouse: 70 mg/kg × 0.08 = **5.6 mg/kg**
(equivalent to approximately 336 mg in a 60-kg subject)

Where 0.08 is the conversion factor to extrapolate the animal dose to the HED based on body surface area.[2] Compared to the HED for the approximate effective dose in the mouse (ie, dose shown to have pharmacological activity in the mouse model), a starting dose of **5**0 mg in Part A will be approximately **7**-fold lower than a dose expected to have pharmacological activity:

$$\frac{5.6 \text{ mg/kg}}{\mathbf{0.83} \text{ mg/kg}} = \mathbf{6.7\text{-fold}}$$

Evaluation of protein binding indicates the unbound drug fraction is 4% in mouse compared to 1.78% in human. The proposed starting dose based on unbound drug is therefore approximately **15** times lower than the effective dose. Although there is no empirical data that defines a pharmacologically inactive dose in the mouse it is considered unlikely that a human starting dose of **5**0 mg would elicit significant pharmacological activity, if any. Furthermore metabolism *in vitro* is notably lower in mouse compared to human. Exposure to parent drug in human is likely to be proportionately less than mouse based on equivalent doses.

Table 4: Proposed Investigational Medicinal Product Dose Levels for Parts A and B

Previously read:

Study Part	Group	Subject Numbers	Treatment Period 1	Treatment Period 2
	A1	0101 – 0108	~~1~~**0** mg or placebo	NA
	A2	0109 – 0116	TBD mg or placebo	NA
	A3[a]	0117 – 0124	TBD mg or placebo (fasted)	TBD mg or placebo (fed)
	A4	0125 – 0132	TBD mg or placebo	NA
Part A	A5	0133 – 0140	TBD mg or placebo	NA
	A6	0141 – 0148	TBD mg or placebo	NA
	A7[b]	0149 – 0156	TBD	TBD
	A8[b]	0157 – 0164	TBD	TBD
	A9[b]	0165 – 0172	TBD	TBD

Now reads:

Study Part	Group	Subject Numbers	Treatment Period 1	Treatment Period 2
	A1	0101 – 0108	**5**0 mg or placebo	NA
	A2	0109 – 0116	TBD mg or placebo	NA
	A3[a]	0117 – 0124	TBD mg or placebo (fasted)	TBD mg or placebo (fed)
	A4	0125 – 0132	TBD mg or placebo	NA
Part A	A5	0133 – 0140	TBD mg or placebo	NA
	A6	0141 – 0148	TBD mg or placebo	NA
	A7[b]	0149 – 0156	TBD	TBD
	A8[b]	0157 – 0164	TBD	TBD
	A9[b]	0165 – 0172	TBD	TBD

Section 3.7: Dose Escalation Stopping Criteria

Previously read:

- The systemic exposure for any individual subject is predicted to exceed a C_{max} of 59,100 ng/mL ~~and/or an AUC_{0-24} of 440,000 ng.h/mL~~ (based on the female monkey kinetics at the NOAEL [350mg/kg/day at Day 28]~~ which is the lowest exposure seen in repeat dose toxicology~~).

Now reads:

- The systemic exposure for any individual subject is predicted to exceed **an AUC_{0-24} of 358,000 ng.hr/mL (based on the female cynomolgus monkey at the intermediate dose level of 175 mg/kg/day at Day 28) in the 28-day oral toxicity study and/or a** C_{max} of 59,100 ng/mL (based on the female **cynomolgus** monkey kinetics at the NOAEL [350 mg/kg/day at Day 28]).

Section 5.3: Randomisation

Previously read:

The randomisation code will be produced by the statistics department at ~~Covance~~ using a computer-generated pseudo-random permutation procedure.

Now reads:

The randomisation code will be produced by the statistics department at **Labcorp Drug Development** using a computer-generated pseudo-random permutation procedure.

Section 5.4: Blinding

Previously read:

The following controls will be employed to maintain the double-blind status of the study:

- The placebo capsules will be identical in appearance to NST-6179.
- The investigator and other members of staff involved with the study will remain blinded to the treatment randomisation code during the assembly procedure.
- The investigator and other members of staff (with the exception of pharmacy staff) involved with the study will remain blinded to the treatment randomisation code during the conduct of the study.
- Interim bioanalytical data will be provided to ~~Covance~~ in a blinded manner.

Now reads:

The following controls will be employed to maintain the double-blind status of the study:

- The placebo capsules will be identical in appearance to NST-6179.
- The investigator and other members of staff involved with the study will remain blinded to the treatment randomisation code during the assembly procedure.
- The investigator and other members of staff (with the exception of pharmacy staff) involved with the study will remain blinded to the treatment randomisation code during the conduct of the study.
- Interim bioanalytical data will be provided to **Labcorp CRU** in a blinded manner.

Appendix 5: Regulatory, Ethical, and Study Oversight Considerations (Data Quality Assurance)

Previously read:

The following data quality steps will be implemented:

- ~~Covance~~ is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds, quality tolerance

thresholds, controls, and mitigation plans will be documented in a project management plan. Additional details of quality checking to be performed on the data may be included in a data management plan.

Now reads:

The following data quality steps will be implemented:

- **Labcorp Drug Development** is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a project management plan. Additional details of quality checking to be performed on the data may be included in a data management plan.

Appendix 5: Regulatory, Ethical, and Study Oversight Considerations (Investigator Documentation Responsibilities)

Previously read:

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to ~~Covance~~ electronically, will be integrated with the subject's eCRF data in accordance with the data management plan.

Now reads:

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to **Labcorp Drug Development** electronically, will be integrated with the subject's eCRF data in accordance with the data management plan.

Version 1 to Version 2

At the request of the Medicines and Healthcare products Regulatory Agency (MHRA), the primary changes in this amendment are:

1. Section 3.5 has been updated to clarify the starting dose is unlikely to be pharmacologically active.
2. Section 3.7 has been updated to base the pharmacokinetic (PK) stopping criteria on the female monkey.

Minor changes:

1. The amendment/version number and date were updated throughout the protocol.

A detailed summary of changes is presented below:

For each section, deleted text is shown in ~~strikethrough~~, and new text is shown in **bold**.

Section 3.5: Selection of Doses in the Study

Evaluation of protein binding indicates the unbound drug fraction is 4% in mouse compared to 1.78% in human. The proposed starting dose based on unbound drug is therefore approximately 9 times lower than the effective dose. Although there is no empirical data that defines a pharmacologically inactive dose in the mouse it is considered unlikely that a human starting dose of 100 mg would elicit significant pharmacological activity, if any. Furthermore metabolism *in vitro* is notably lower in mouse compared to human. Exposure to parent drug in human is likely to be proportionately less than mouse based on equivalent doses.

Section 3.7: Dose Escalation Stopping Criteria

Previously read:

- The systemic exposure for any individual subject is predicted to exceed a C_{max} of ~~69,400~~ ng/mL and/or an AUC_{0-24} of 1~~,090,000~~ ng.h/mL (~~ie, systemic exposure will be no greater than the C_{max} and AUC_{0-24} at the NOAEL with the lowest HED [rat]~~).

Now reads:

- The systemic exposure for any individual subject is predicted to exceed a C_{max} of **59,100** ng/mL and/or an AUC_{0-24} of **440,000** ng.h/mL (**based on the female monkey kinetics at the NOAEL [350mg/kg/day at Day 28] which is the lowest exposure seen in repeat dose toxicology**).