Protocol

A Phase 1, Open-label Study to Evaluate the Effects of NST-1024 on the Pharmacokinetics of Caffeine, Flurbiprofen, Omeprazole, Metoprolol, and Midazolam in Healthy Subjects

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INVESTIGATOR AGREEMENT

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

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SYNOPSIS

Study Title

A Phase 1, Open-label Study to Evaluate the Effects of NST-1024 on the Pharmacokinetics of Caffeine, Flurbiprofen, Omeprazole, Metoprolol, and Midazolam in Healthy Subjects

Objectives and Endpoints

Objectives	Endpoints
Primary:	
• to evaluate the effects of NST-1024 on the PK of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam in healthy male and female subjects	• primary PK parameters: $AUC_{0\text{-}inf}$, $AUC_{0\text{-}tlast}$, and C_{max}
Secondary:	
• to assess the safety and tolerability of NST-1024 in healthy subjects	• incidence and severity of AEs • incidence of laboratory abnormalities, based on haematology, clinical chemistry, and urinalysis test results • 12-lead ECG parameters • vital signs measurements • physical examinations

Abbreviations: AE = adverse event; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}tlast}$ = area under the concentration-time curve from time 0 to the time of the last quantifiable concentration; C_{max} = maximum observed concentration; ECG = electrocardiogram; PK = pharmacokinetic(s).

Study Design

This will be a Phase 1, open-label study to evaluate the effects of NST-1024 on the pharmacokinetics (PK) of caffeine (and paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and 1-hydroxymidazolam) in healthy male and female subjects. Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Subjects will be admitted into the study site on Day -1 and be confined until discharge on Day 3. Subjects will return for check-in on Day 7 and be confined until discharge on Day 23. Subjects will return to the study site for a follow-up visit 7 to 10 days after the last dose.

Number of Subjects

Up to 21 subjects will be enroled in order that 18 subjects complete the study.

Diagnosis and Main Criteria for Inclusion

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

All subjects will receive each of the following treatments:

- Day 1: single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam
- Days 8 to 22: oral doses of 200 mg NST-1024 once daily multiple-dose regimen
- Day 8: single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024
- Day 21: single oral dose of 100 mg caffeine and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024.

All doses of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam, including dosing occasions when coadministered with NST-1024, will be given in a fasted state.

Duration of Subject Participation in the Study

Planned screening duration: approximately 4 weeks

Planned study duration (screening to follow-up): approximately 9 weeks.

Statistical Methods

Pharmacokinetics

Pharmacokinetic parameters will be determined from the plasma concentrations of caffeine (and its metabolite paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and its metabolite 1-hydroxymidazolam) using standard noncompartmental methods.

The primary PK parameters are area under the concentration-time curve (AUC) from time 0 extrapolated to infinity ($AUC_{0\text{-}inf}$), AUC from time 0 to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$), and maximum observed concentration (C_{max}). Additional PK parameters may be calculated. All other PK parameters will be regarded as secondary and will not be subject to inferential statistical analysis.

A linear mixed-model analysis will be applied to analyse the log-transformed primary PK parameters. The model will assume a fixed effect for treatment and a random effect for subject. Estimates of geometric least squares mean ratios together with the corresponding 90% confidence intervals will be derived for each of the parameters separately as follows:

- Probe drugs coadministered with NST-1024 (profile Day 8) (test) versus probe drugs alone (profile Day 1) (reference). This comparison will be done for caffeine,

paraxanthine, flurbiprofen, omeprazole, metoprolol, midazolam, and 1-hydroxymidazolam analytes separately

- Probe drugs coadministered with NST-1024 (profile Day 21) (test) versus probe drugs alone (profile Day 1) (reference). This comparison will be done for caffeine, paraxanthine, midazolam, and 1-hydroxymidazolam analytes separately.

Safety

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

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AUC	area under the concentration-time curve
$AUC_{0\text{-inf}}$	area under the concentration-time curve from time 0 extrapolated to infinity
$AUC_{0\text{-tlast}}$	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration
CFR	Code of Federal Regulations
C_{max}	maximum observed concentration
CRO	contract research organisation
CRU	clinical research unit
CV	cardiovascular
CYP	cytochrome P450
DDI	drug-drug interaction
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EPA	eicosapentaenoic acid
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
HDL-C	high-density lipoprotein cholesterol
HTG	hypertriglyceridemia
IB	investigator's brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IMP	investigational medicinal product
IRB	institutional review board
LDL-C	low-density lipoprotein cholesterol
PK	pharmacokinetic(s)
qd	once daily
QTcF	QT interval corrected for heart rate using Fridericia's method
SAE	serious adverse event
TEAE	treatment-emergent adverse event
TG	triglyceride

1. INTRODUCTION

Refer to the investigator's brochure (IB)[1] for detailed information concerning the available pharmacology, toxicology, drug metabolism, clinical studies, and adverse event (AE) profile of the investigational medicinal product (IMP).

1.1. Overview

NorthSea Therapeutics is developing NST-1024, a novel, orally administered, highly potent, semi-synthetic fatty acid for the treatment of severe hypertriglyceridemia (HTG), mixed dyslipidemia, and hypercholesterolemia. In severe HTG, the primary goal of therapy is to prevent development of acute pancreatitis by lowering triglyceride (TG) levels. Mixed dyslipidemia is characterised as elevated low-density lipoprotein cholesterol (LDL-C) and TG, often accompanied by low high-density lipoprotein cholesterol (HDL-C) levels. Elevations of TG signals lead to an increased risk of atherosclerotic cardiovascular (CV) events even when the LDL-C is well controlled by statin therapy. NST-1024 is a semi-synthetic, structurally engineered eicosapentaenoic acid (EPA) derivative. The structural modifications are designed to potentiate the pharmacological effects of naturally occurring EPA by (a) targeting the intestine and liver by limiting peripheral distribution and (b) remaining as a free acid by reducing both the incorporation into complex lipids and metabolism via β-oxidation, thereby increasing availability for fatty-acid sensitive signalling pathways. The nonclinical experience with NST-1024 thus far indicates large reductions in both TG and non-HDL-C in conjunction with a marked increase in HDL-C. In addition to beneficial effects on lipid and lipoprotein metabolism, significant improvements in glycaemic control are observed in diabetic mice. It has also been demonstrated that NST-1024 depletes hepatic cholesterol, which in turn increases hepatic uptake of plasma cholesterol and TG without a compensatory increase in hepatic secretion of very-low-density lipoprotein-TG. NST-1024 is a liquid, and is dosed orally, with the current dosage form being a red, soft gelatin capsule containing the active pharmaceutical ingredient in a self-emulsifying drug delivery system. Current formulations contain 10 or 100 mg NST-1024 formulated in polysorbate 80 and medium-chain TGs with dosing anticipated to be once daily (qd).

1.2. Nonclinical Pharmacokinetic Drug Interactions

Pharmacokinetic (PK) drug interactions were assessed in the Covance 8424700 study which was conducted to measure the extent of induction of specific human cytochrome P450 (CYP) enzymes (CYP1A2, CYP2B6, and CYP3A4) following exposure of human hepatocytes from 3 donors to NST-1024, and to compare the effects of the test article with those of prototypical inducers. Results indicated that NST-1024 up to 50 μM, was present in the incubation medium for 24 hours, was not cytotoxic, and induced all of the tested CYP enzymes (CYP1A2, CYP2B6, and CYP3A4/5) in at least 1 donor as measured by mRNA and CYP activity levels.

A further study (Covance 8424669) was conducted to characterise the in vitro inhibitory potential of NST-1024 on the activities of the following human liver CYP enzymes: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5. NST-1024 (0.3 to 200 μM or 300 μM) exhibited direct inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 (testosterone 6β-hydroxylase), and CYP3A4/5

(midazolam 1'-hydroxylase). NST-1024 exhibited no metabolism-dependent inhibition of the CYP enzymes tested. Under the same experimental conditions, the known direct and metabolism-dependent inhibitors of CYP enzymes exhibited marked inhibition of the corresponding CYP enzymes. Overall, the CYP inhibition-based drug-drug interaction (DDI) assessment for NST-1024 using a basic model indicated a potential for DDI is likely to occur with CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 (testosterone), and CYP3A4/5 (midazolam) probe drugs.

1.3. Summary of Clinical Experience

1.3.1. Safety

As of the date of this final protocol (Version 1), a total of 94 subjects have been exposed to either NST-1024 or matched placebo at doses up to 400 mg as a single dose and up to 200 mg in repeat dosing for 14 days. The study is ongoing and remains blinded, but an analysis of the treatment-emergent AEs (TEAEs) was performed as part of the most recent dose escalation review. A total of 61 TEAEs in 38 subjects were reported during the study period, of which 7 were assessed as related and >85% were assessed as mild severity. All reported events resolved during the study period. There were no severe or serious TEAEs reported during the study period. The most common TEAE (>2 subjects) reported during the study period were headache (n=10; 3 related), loose stools (n=3; 2 related), and coronavirus disease 2019 positive test (n=3; none related). There were 7 TEAEs assessed as drug-related, all were considered mild and included headache (n=3), loose stools (n=2), nausea (n=1) and abdominal pain (n=1). There were no clinically significant changes in laboratory parameters suggestive of a safety signal. Vital signs and electrocardiogram (ECG) parameters remained stable throughout study drug dosing. In summary, NST-1024 appears to be safe and well tolerated at single dose up to 400 mg and repeated dose of 200 mg up to 14 days.

1.3.2. Pharmacokinetics

The PK data of NST-1024 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 10, 30, 50, 60, 200, and 400 mg were tested under fasted conditions. Single dosing was performed with a high-fat meal at the 30 and 100 mg dosing levels. Repeat dosing was performed following a standard breakfast at the 10, 30, 60, and 200 mg dosing levels. Standard PK parameters, accumulation ratio, and dose proportionality were evaluated as available. There was no significant accumulation noted between single and repeated once daily dosing under fed conditions. Dose proportionality for both area under the concentration time curve (AUC) and maximum observed concentration (C_{max}) was generally observed with increasing doses over the range of 10 to 60 mg with less than proportional increases in exposure observed following 200 mg and 400 mg single doses in the fasted state. Slightly more than proportional increases in exposure relative to increasing dose were observed following multiple doses in the fed state. The key PK parameters for doses of NST-1024 evaluated to date are presented in Table 1 and Table 2.

Table 1: Key Pharmacokinetic Parameters of NST-1024 Single Doses Evaluated to Date in Study NST-1024-01

Parameter (Units)	10 mg (Single, Fasted)	30 mg (Single, Fasted)	30 mg (Single, Fed**)	50 mg (Single, Fasted)	60 mg (Single, Fasted)	100 mg (Single, Fed**)	200 mg (Single, Fasted)	400 mg (Single, Fasted)
Dosing Group	A2	A3		A1	A4	A5	A6	A7
	N=6	N=6	N=6	N=6	N=6	N≤6	N≤6	N=6
AUC_{0-24} (ng*h/mL)	1360 (23.5)	3800 (15.9)	3530 (19.5)	8610 (38.3)	8160 (22.1)	13 200 (40.5)	22 100 (26.0)	38400 (29.6)
C_{max} (ng/mL)	374 (23.0)	1140 (13.7)	665 (18.7)	2310 (32.3)	2530 (26.6)	3010 (67.3)	5090 (35.0)	8860 (32.7)
t_{max} [a] (h)	1.00 (1.00–1.50)	1.25 (1.00-2.00)	3.00 (2.00–3.00)	1.50 (1.00–2.00)	1.00 (1.00-1.50)	1.00 (0.500-3.00)	2.00 (1.00-3.00)	3.00 (2.00-4.00)
$t_{1/2}$ (h)	3.58 (17.2)	4.32 (13.3)	7.15 (25.2)	4.71 (15.7)	6.15 (23.4)	6.46 (24.0)	5.47 (38.3)	5.87 (23.2)

**High-fat meal administered at 30 mg or 100 mg for food effect assessment during single ascending dose phase.
[a] Median (min-max) presented.
NA = Not applicable.

Table 2: Key Pharmacokinetic Parameters of NST-1024 Repeat Doses Evaluated to Date in Study NST-1024-01

Parameter (Units)	10 mg (Single, Fed*)	10 mg (Repeat, Fed*)	30 mg (Single, Fed*)	30 mg (Repeat, Fed*)	60 mg (Single, Fed*)	60 mg (Repeat, Fed*)	200 mg (Single, Fed*)	200 mg (Repeat, Fed*)
Dosing Group	**B1**		**B2**		**B3**		**B4**	
	N=8	**N=8**	**N=8**	**N=8**	**N=6**	**N=6**	**N≤8**	**N≤8**
AUC_{0-24} (ng*h/mL)	**898 (26.4)**	**1050 (19.3)**	**3410 (27.1)**	**3530 (20.6)**	**5930 (26.9)**	**6770 (27.3)**	**29100 (26.1)**	**29500 (21.8)**
C_{max} (ng/mL)	**181 (22.5)**	**190 (25.3)**	**665 (23.1)**	**599 (27.7)**	**1030 (18.2)**	**1390 (23.6)**	**6510 (44.5)**	**6080 (31.9)**
C_{min} (ng/mL)	**NA**	**2.96 (26.4)**	**NA**	**9.94 (52.9)**	**NA**	**16.1 (83.2)**	**NA**	**84.7 (53.4)**
t_{max} [a] (h)	**2.50 (1.00-4.00)**	**2.00 (1.50-4.00)**	**2.00 (1.00-4.00)**	**2.50 (1.50-4.00)**	**2.00 (1.50-4.00)**	**1.50 (1.00-1.50)**	**4.00 (1.50-6.00)**	**4.00 (2.00-6.00)**
$t_{1/2}$ (h)	**3.52 (35.3)**	**5.16 (17.7)**	**3.90 (24.5)**	**6.76 (22.3)**	**3.81 (12.3)**	**5.65 (31.1)**	**4.06 (17.4)**	**5.77 (23.4)**

* Standard breakfast administered for MAD cohort, with repeat dose PK assessed on Day 14.
[a] Median (min-max) presented.
NA = Not applicable.

1.4. Study Rationale

The objective of DDI studies is to determine whether potential interactions between an investigational drug and other drugs exist. Drug-drug interaction studies have an important role in drug development, and this study is being performed as part of the development program for NST-1024.Given the potential for NST-1024 to cause induction and/or inhibition of CYP enzymes as discussed in Section 1.2, a DDI study is warranted to investigate the potential for CYP induction and/or inhibition clinically. NST-1024 may have the potential to raise plasma concentrations of drugs that are probe drugs of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 or lower plasma concentrations that are metabolised by CYP1A2 or CYP3A4. This study is designed to determine the effect of NST-1024 on the PK of caffeine (CYP1A2), flurbiprofen (CYP2C9), omeprazole (CYP2C19), metoprolol (CYP2D6), and midazolam (CYP3A4). Caffeine and midazolam on Day 8 will assess the inhibition potential of CYP1A2 and 3A4 and on Day 21 will assess the induction potential of CYP1A2 and 3A4. The effects of NST-1024 on the PK of other CYP450 probe drugs may be explored in separate clinical studies. Caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam are commonly used as index probe drugs for CYP450 enzymes in DDI studies.

1.5. Benefit-risk Assessment

Healthy 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 treatments, 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 AEs associated with NST-1024 may be found in the IB.[1]

The side effects of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam are well characterised and generally predictable. The risks of clinically significant side effects related to the doses of probe drugs used in this trial are considered minimal. Dosing will only occur while subjects are resident at the clinical research unit (CRU), under medical supervision. Information about the known and expected risks of the probe drugs may be found in their respective prescribing information.

2. OBJECTIVES AND ENDPOINTS

Table 3 shows the objectives and endpoints of the study.

Table 3: Objectives and Endpoints

Objectives	Endpoints
Primary:	
• to evaluate the effects of NST-1024 on the PK of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam in healthy male and female subjects	• primary PK parameters: $AUC_{0\text{-}inf}$, $AUC_{0\text{-}tlast}$, and C_{max}
Secondary:	
• to assess the safety and tolerability of NST-1024 in healthy subjects	• incidence and severity of AEs • incidence of laboratory abnormalities, based on haematology, clinical chemistry, and urinalysis test results • 12-lead ECG parameters • vital signs measurements • physical examinations

Abbreviations: AE = adverse event; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 extrapolated to infinity; $AUC_{0\text{-}tlast}$ = area under the concentration-time curve from time 0 to the time of the last quantifiable concentration; C_{max} = maximum observed concentration; ECG = electrocardiogram; PK = pharmacokinetic(s).

3. INVESTIGATIONAL PLAN

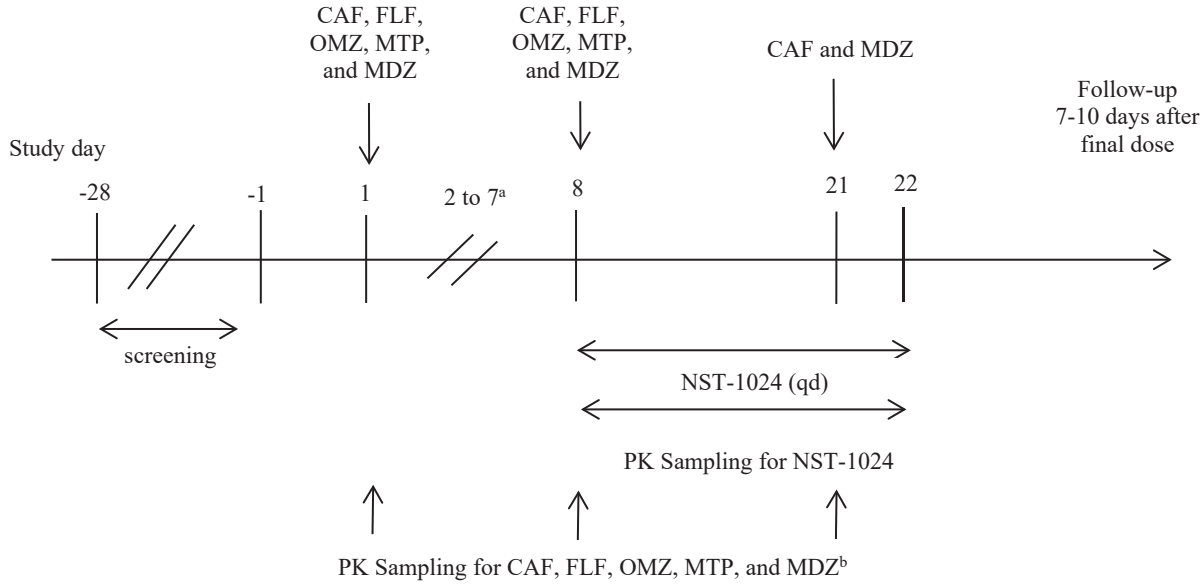
3.1. Overall Study Design and Plan

This will be a Phase 1, open-label study to evaluate the effects of NST-1024 on the PK of caffeine (and paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and 1-hydroxymidazolam) in healthy male and female subjects. Up to 21 subjects will be enroled to ensure that 18 subjects complete the study. All subjects will receive each of the following treatments:

- Day 1: single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam
- Days 8 to 22: oral doses of 200 mg NST-1024 qd multiple-dose regimen
- Day 8: single oral dose of 100 mg caffeine, 50 mg flurbiprofen, 20 mg omeprazole, 100 mg metoprolol, and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024
- Day 21: single oral dose of 100 mg caffeine and 2.5 mg midazolam coadministered with an oral dose of 200 mg NST-1024.

An overview of the study design is shown in Figure 1.

Figure 1: Study Schematic



Abbreviations: CAF = caffeine; FLF = flurbiprofen; MDZ = midazolam; MTP = metoprolol; OMZ = omeprazole; PK = pharmacokinetic(s); qd = once daily
[a] Subjects will discharge on Day 3 and return for check-in on Day 7.
[b] Pharmacokinetic sampling for CAF, FLF, MDZ, MTP, and OMZ on Days 1 and 8: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose. Pharmacokinetic sampling for CAF and MDZ on Day 21: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Subjects will be admitted into the study site on Day -1 and be confined until discharge on Day 3. Subjects will return for check-in on Day 7 and be confined until discharge on Day 23. Subjects will return to the study site for a follow-up visit 7 to 10 days after the last dose.

The total duration of study participation for each subject (from screening through follow-up visit) is anticipated to be approximately 61 days.

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).

A Schedule of Assessments is presented in Appendix 6.

3.2. Discussion of Study Design

The fixed sequence design used in this study is typical for interaction studies where a relatively small number of subjects are required, because it allows intrasubject comparisons and reduces intersubject variability. This study will be open-label because the study endpoints are not considered subjective.

This study will investigate the potential effects of NST-1024 on those hepatic enzymes that demonstrated inhibition in human liver microsomes in vitro: CYP1A2 (caffeine), CYP2C9 (flurbiprofen), CYP2C19 (omeprazole), CYP2D6 (metoprolol), and CYP3A4 (midazolam). The study will also investigate the induction potential of CPY1A2 and CYP3A4. The probe drugs chosen for this study are principally cleared via metabolism by the indicated CYP and are regarded as a prototypical probe drugs of that CYP. The probe drugs are frequently used as probe drugs for investigation of DDIs mediated through inhibition or induction of the relevant CYP. The PK after a single dose of NST-1024 will investigate inhibition potential; PK after multiple doses of NST-1024 to investigate induction potential.

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

3.3. Selection of Doses in the Study

Single and multiple doses of 200 mg of NST-1024 have been shown to be safe and well tolerated in healthy subjects in Study NST-1024-01. A single dose of 200 mg is considered sufficient to assess the inhibition potential of the CYP enzymes while 15 days (Days 8 to 22 inclusive) of repeat dosing with NST-1024 is considered sufficient for full induction potential of the CYP enzymes. Although single doses of 400 mg are safe and well tolerated, a lower dose of 200 mg was selected for both the inhibition and induction phases of this study. The exposure (both AUC and C_{max}) of 200 mg multiple dose is significantly lower than the no-observed-adverse-effect level from the dog toxicology study (75 mg/kg/day), providing an adequate safety margin in case of significant increase in NST-1024 concentration is observed.

The doses of caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam were selected as they are the most sensitive probe drugs and there is a large body of information on the relative contribution of specific CYP pathways to their overall elimination, their appropriate dosing regimens, safety profiles, and anticipated interaction effects when coadministered with strong CYP inhibitors or inducers.

4. SELECTION OF STUDY POPULATION

4.1. Inclusion Criteria

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, 12-lead ECG, vital signs measurements, and clinical laboratory evaluations (congenital nonhaemolytic hyperbilirubinemia [eg, suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable) at screening and/or check-in and from the physical examination at 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

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, CV, gastrointestinal, neurological, respiratory, endocrine, or psychiatric disorder, as determined by the investigator (or designee).
2. History of febrile illness within 1 week prior to the first dose.
3. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the investigator (or designee).
4. 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).
5. Aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, or total bilirubin >1.2 × the upper limit of normal at Screening or (first) Check-in, confirmed by 1 repeat if necessary.
6. Confirmed (eg, 2 consecutive measurements) systolic blood pressure >160 or <80 mmHg, diastolic blood pressure >90 or <45 mmHg, and pulse rate >100 or <40 beats per minute.
7. Positive hepatitis panel and/or positive human immunodeficiency virus test (Appendix 2).

Prior/concomitant therapy

8. Administration of a coronavirus disease 2019 vaccine in the past 30 days prior to dosing.
9. Use or intend to use any medications/products known to alter drug absorption, metabolism, or elimination processes, including over-the-counter and herbal medication, within 30 days prior to dosing, unless deemed acceptable by the investigator (or designee).
10. 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).
11. 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).

12. Use or intend to use any nonprescription medications (except paracetamol, up to 2 g per day)/products including vitamins, dietary supplements, minerals, and phytotherapeutic/plant-derived preparations within 7 days prior to check-in, unless deemed acceptable by the investigator (or designee). Herbal supplements must be discontinued at least 28 days prior to the first dose of study drug.

Prior/concurrent clinical study experience

13. Participation in a clinical study involving administration of an investigational drug (new chemical entity) in the past 90 days prior to dosing.

Diet and lifestyle

14. Alcohol consumption of >21 units (males) and >14 units (females) 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. Subjects must also be willing to not donate blood for 3 months after the follow-up visit.
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 would 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 prior to the first dosing occasion. Assignment of subject numbers will be in ascending order and no numbers will be omitted (eg, Subjects 0101, 0102, 0103). 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 101).

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.

Subjects who are initially found to be ineligible may be rescreened at the discretion of the investigator.

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 from dosing 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)
- noncompliance 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. If a subject is withdrawn from the study, efforts will be made to perform all follow-up assessments, if possible (Appendix 6). Other applicable safety-related 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
- cancelation of drug development.

5. STUDY TREATMENTS

5.1. Description, Storage, Packaging, and Labelling

NST-1024 will be supplied as liquid in soft gelatin capsules, 100 mg 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. NST-1024 will be provided in bulk bags and stored according to the instructions on the label.

The investigator will commercially source caffeine (100 mg in 2 × 50-mg tablets), flurbiprofen (50-mg tablet), omeprazole (20-mg capsule), metoprolol (100-mg coated tablet), and midazolam (2.5-mg solution). The tablets and capsules will be provided in blisters and the solution will be provided in syringes. The probe drugs will be stored according to the manufacturer's instructions.

Investigational medicinal products will be stored at the study site in a location that is locked with restricted access.

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

Each dose of NST-1024 and probe drugs will be administered orally with approximately 240 mL of room temperature water. When NST-1024 and probe drugs are administered concurrently, only 240 mL of room temperature water will be administered for all drugs, though additional water (up to another approximately 240 mL) is permissible for the Day 1 dose and Day 8 dose when the total number of coadministered drugs is relatively high. Water restrictions around dosing around dosing are provided in Section 6.2.

All doses will be given in a fasted state.

Subjects will be dosed 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

This is a nonrandomised study. The study has a fixed treatment sequence.

5.4. Blinding

This is an open-label study.

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.
- At each dosing occasion, a predose and postdose inventory of NST-1024 and caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam will be performed.

5.6. Drug Accountability

The investigator (or designee) will maintain an accurate record of the receipt of NST-1024 capsules 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 NST-1024 capsules will be returned to the sponsor or disposed of by the study site, per the sponsor's written instructions.

Caffeine, flurbiprofen, omeprazole, metoprolol, and midazolam will also be subject to accountability procedures, and the study site staff will destroy unused supplies of probe drugs at the end of the study.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Subjects will refrain from use of any prescription or nonprescription 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.

6.2. Diet

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

On the days with PK assessments (Days 1, 8, and 21), the subjects will be fasted overnight (at least 8 hours) prior to dosing and refrain from consuming water from 1 hour predose until 2 hours postdose, excluding the amount of water consumed at dosing. Food is allowed from 4 hours postdose. At all other times during the study, subjects may consume water ad libitum.

All doses will be given in a fasted state.

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 after the follow-up visit.

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-1024 assay)
- pulse oximetry
- vital signs
- 12-lead ECGs
- any other procedures.

7.1. Pharmacokinetic Assessments

7.1.1. Pharmacokinetic Blood Sample Collection and Processing

Blood samples (approximately 1 × 1 mL for NST-1024, 1 × 4 mL for caffeine [and paraxanthine] and midazolam [and 1-hydroxymidazolam], 1 × 4 mL for flurbiprofen, omeprazole, and metoprolol) will be collected by venepuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6. Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

7.1.2. Analytical Methodology

Plasma concentrations of NST-1024, caffeine (and paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and 1-hydroxymidazolam) will be determined using a validated analytical procedure. Specifics of the analytical method 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 serious AEs 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, to 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.

Subjects will be asked to provide urine samples for 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, 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.

Blood pressure will be taken in triplicate at approximately 2-minute intervals on Day 1, predose, and as single measurements at all other times. All other measurements will be performed singly, and repeated once if outside the relevant clinical reference range.

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

7.2.4. 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:

- QT interval corrected for heart rate using Fridericia's method (QTcF) >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.

7.2.5. Physical Examination

A full physical examination or symptom-directed physical examination will be performed at the timepoints specified in the Schedule of Assessments in Appendix 6.

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

Up to 21 subjects will be enroled in order that 18 complete 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-1024, caffeine, flurbiprofen, omeprazole, metoprolol, or midazolam) and have at least 1 valid PK concentration.

8.2.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (NST-1024, caffeine, flurbiprofen, omeprazole, metoprolol, or midazolam).

8.2.3. All Subjects Population

The all subjects population will include all subjects who signed the ICF and have any study assessment recorded in the database per the protocol.

8.3. Pharmacokinetic Analyses

Pharmacokinetic parameters will be determined from the plasma concentrations of caffeine (and its metabolite paraxanthine), flurbiprofen, omeprazole, metoprolol, and midazolam (and its metabolite 1-hydroxymidazolam) using standard noncompartmental methods.

The primary PK parameters are AUC from time 0 extrapolated to infinity ($AUC_{0\text{-}inf}$), AUC from time 0 to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$), and C_{max}. Additional PK parameters may be calculated. All other PK parameters will be regarded as secondary and will not be subject to inferential statistical analysis. Full details of PK parameters will be presented in the statistical analysis plan for this study.

A linear mixed-model analysis will be applied to analyse the log-transformed primary PK parameters. The model will assume a fixed effect for treatment and a random effect for subject. Estimates of geometric least squares mean ratios together with the corresponding 90% confidence intervals will be derived for each of the parameters separately as follows:

- Probe drugs coadministered with NST-1024 (profile Day 8) (test) versus probe drugs alone (profile Day 1) (reference). This comparison will be done for caffeine,

paraxanthine, flurbiprofen, omeprazole, metoprolol, midazolam, and 1-hydroxymidazolam analytes separately

- Probe drugs coadministered with NST-1024 (profile Day 21) (test) versus probe drugs alone (profile Day 1) (reference). This comparison will be done for caffeine, paraxanthine, midazolam, and 1-hydroxymidazolam analytes separately.

8.4. Safety Analysis

All AEs will be listed and TEAEs will be summarised. Each AE will be coded using the Medical Dictionary for Regulatory Activities. Observed values for clinical laboratory test data, 12-lead ECGs, and vital signs, and physical examination findings will be listed.

8.5. Interim Analysis

No interim analyses are planned for this study.

9. REFERENCES

1. NST-1024 [Investigator's Brochure]. Amsterdam, The Netherlands: NorthSea Therapeutics B.V.; Version 1.0, 23 June 2020.

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 4-category system according to the following guidelines:

- **Not Related**: The AE is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Unlikely Related**: The temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly Related**: The AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the subject's clinical state or the study procedures/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 possibly related or 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 or unlikely 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 for an unapproved IMP).

Serious Adverse Events

A serious AE (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 possibly related to the study treatment, will be reported to the sponsor.

<u>Definition of Life-threatening</u>

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. If anything untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'nonserious' 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 CRU standard operating procedure 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/ethics committee, Medicines and Healthcare products Regulatory Agency, 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	Haematocrit	Blood
Albumin	Haemoglobin	Glucose
Alkaline phosphatase	Mean cell haemoglobin	Ketones
Aspartate aminotransferase	Mean cell haemoglobin concentration	pH
Bilirubin (total and direct)	Mean cell volume	Protein
Calcium	Platelet count	Specific gravity
Chloride	Red blood cell (RBC) count	Urobilinogen
Cholesterol	White blood cell (WBC) count	Microscopic examination
Creatinine	WBC differential:	
Gamma glutamyl transferase	Basophils	
Glucose	Eosinophils	
Inorganic phosphate	Lymphocytes	
Potassium	Monocytes	
Sodium	Neutrophils	
Total protein		
Uric acid		
Serology:	**Drug screen:**	**Hormone panel - females only [a]:**
Hepatitis B surface antigen	Including but not limited to:	Follicle-stimulating hormone
Hepatitis C antibody	Amphetamines/methamphetamines	Serum pregnancy test (human chorionic gonadotropin)[b]
Human immunodeficiency virus (HIV-1 and HIV-2) antibodies and p24 antigen	Barbiturates	
	Benzodiazepines	
	Cocaine (metabolite)	
	Methadone	
	Phencyclidine	
	Opiates	
	Tetrahydrocannabinol/ cannabinoids	
	Cotinine test	
	Alcohol breath test	

[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

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	6	45
Serology	3.5	1	3.5
NST-1024 PK	1	15	15
Caffeine (and paraxanthine) and midazolam (and 1-hydroxymidazolam) PK	4	42	168
Flurbiprofen, omeprazole, and metoprolol PK	4	28	112
Total:			343.5

Abbreviations: PK = pharmacokinetic(s)
[a] Includes pregnancy and follicle-stimulating hormone tests.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 550 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 Nonchildbearing 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:** female 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, selective oestrogen receptor modulators, or chemotherapy. Females on hormone replacement therapy with FSH levels <40 mIU/mL may be included at the discretion of the investigator. Women 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 nonchildbearing potential will not be required to use contraception. For female subjects of childbearing potential, 2 methods (1 primary acceptable and 1 secondary method) of birth control are required 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
- 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).

A secondary (barrier) method of contraception would include:

- condom with spermicide.

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 in conjunction with partners of childbearing potential use the following contraceptive measure:

- condom and spermicide (even if subject has a history of vasectomy).

In addition, use a second method of acceptable contraception from check-in until 90 days after discharge. 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 intrauterine device.

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

A subject who practices total abstinence is required to identify contraceptive methods he/she will use in the event of sexual activity. Periodic abstinence (eg, calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.

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 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, 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 nonsubstantial 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 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 reconsented 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 investigator 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 enroled 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

Study Procedures	Screening	Day -1	Days 1 to 23	Follow-up (7 to 10 days post final dose)
Informed consent	X			
Inclusion/exclusion criteria	X	X	Day 1: predose	
Demographic data	X			
Medical history	X	X[a]		
Urinary drug screen	X	X	Day 7	
Urine cotinine test	X	X	Day 7	
Alcohol breath test	X	X	Day 7	
Serology	X			
Pregnancy test[b]	X	X	Day 7	
FSH[c]	X			
Height and body weight	X			
Study residency:				
Check-in		X	Day 7	
Check-out			Days 3 and 23 (24 hours post final dose)	
Nonresidential visit	X			X
Study treatment administration:				
NST-1024			Days 8 to 22 (inclusive)	
Omeprazole, flurbiprofen, metoprolol			Days 1 and 8	
Caffeine and midazolam			Days 1, 8, and 21	
Pharmacokinetics[d]:				
NST-1024 blood sampling (15 samples/subject)			Days 8 to 22: predose	
Flurbiprofen, omeprazole, metoprolol blood sampling (28 samples/subject)			Days 1 and 8: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose	
Caffeine (and paraxanthine) and midazolam (and 1-hydroxymidazolam) blood sampling (42 samples/subject)			Days 1, 8, and 21: predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, and 48 hours postdose	
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X

Schedule of Assessments

Study Procedures	Screening	Day -1	Days 1 to 23	Follow-up (7 to 10 days post final dose)
Prior/concomitant medication monitoring	X	X	Ongoing	X
Clinical laboratory evaluations	X	X	Days 8, 14, and 21	X
Blood pressure, pulse rate[e]	X		Days 1, 8, and 21: predose, 0.5, 1, 2, 3, 4, 8, 12, 24, and 48 hours postdose Days 15 and 18: predose	X
Oral body temperature	X		Days 1, 8, and 21: predose, 0.5, 1, 2, 3, 4, 8, 12, 24, and 48 hours postdose Days 15 and 18: predose	X
Pulse oximetry			Days 1, 8, and 21: -1 to 6 h postdose with source data collected at predose, 1, 2, 3, 4, 5, and 6 hours postdose	
12-lead ECG[f]	X		Days 1, 8, and 21: predose, 1, 4, and 24 hours postdose Days 15, and 18: predose	X
Physical examination[g]		X	Symptom-directed physical examination when required	X

Abbreviations: ECG = electrocardiogram; FSH = follicle-stimulating hormone
[a] Interim medical history
[b] Performed for all female subjects 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 for all female subjects
[d] Predose pharmacokinetic blood samples should be taken within 30 minutes prior to dosing.
[e] Blood pressure will be taken in triplicate at approximately 2-minute intervals on Day 1, predose, and as single measurements at all other times. On Day 1 predose assessments should be performed within 2 hours prior to dosing. On all other days of dose administration, predose assessment should be performed within 30 minutes prior to dosing.
[f] 12-lead ECGs will be taken in triplicate at screening at approximately 2-minute intervals and as single measurements at all other times. On Day 1 predose assessments should be performed within 2 hours prior to dosing. On all other days of dose administration, predose assessment should be performed within 30 minutes of dosing.
[g] A full physical examination will be conducted at check-in. Symptom-directed physical examinations will be conducted thereafter.