Protocol

A Randomised, Partially Double-blind, Placebo- and Positive-controlled, 4-way Crossover Study to Evaluate the Effect of Icosabutate (NST-4016) on the QT/QTc Interval in Healthy Subjects

Protocol Status: Final
Protocol Date: 11 April 2018

Investigational Product: icosabutate (NST-4016)

Protocol Reference Number: NST-01
Covance Study Number: 8383557
EudraCT Number: 2018-000395-14

Sponsor:
NorthSea Therapeutics BV
Gooimeer 2-35
1411, DC Naarden
The Netherlands

Study Site:
Covance Clinical Research Unit (CRU) Ltd.
Springfield House
Hyde Street
Leeds, LS2 9LH
United Kingdom

Sponsor Signatory:
Dr. Patrick Round, MBBS FFPM

Principal Investigator:
Dr. Ashley Brooks, MBChB

SPONSOR APPROVAL

I have read the protocol and approve it:

15 APRIL 2018

Dr. Patrick Round, MBBS FFPM
Chief Medical Officer
NorthSea Therapeutics BV

Date

INVESTIGATOR AGREEMENT

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

Dr. Ashley Brooks, MBChB
Principal Investigator

16 APR 2018
Date

STUDY IDENTIFICATION

Sponsor	NorthSea Therapeutics BV Gooimeer 2-35 1411, DC Naarden The Netherlands
Sponsor's Study Contact and Medical Contact	Dr. Patrick Round, MBBS FFPM Chief Medical Officer NorthSea Therapeutics BV Gooimeer 2-35 1411, DC Naarden The Netherlands Tel: +44 (0)7979 502 770 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. Ashley Brooks, MBChB Principal Investigator Covance CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0)113 301 3521 Tel (after hours): +44 (0)113 301 3500
Project Physician	Dr. Hamzah Malik, MD Clinical Research Physician Covance CRU Ltd. Springfield House Hyde Street Leeds, LS2 9LH United Kingdom Tel: +44 (0)113 301 3656
Clinical Laboratory	Covance Clinical Pathology Services Otley Road Harrogate, HG3 1PY United Kingdom
Bioanalytical Laboratory	Covance Laboratories Europe (CLE) Otley Road Harrogate, HG3 1PY United Kingdom

Central Electrocardiogram Laboratory	iCardiac Technologies, Inc. (an ERT company) 150 Allens Creek Rd Rochester New York 14618 United States
Regulatory and Monitoring	QRC Consultants Ltd Silvaco Technology Centre Compass Point Business Park Stocks Bridge Way Saint Ives PE27 5JL United Kingdom
Pharmacovigilance	Bionical Emas 63-65 Knowl Piece Wilbury Way Hitchin, Hertfordshire SG4 0TY United Kingdom
Statistician	Izabela Antys, BSc MSc GradStat Covance Biometrics

SYNOPSIS

Title of study: A Randomised, Partially Double-blind, Placebo- and Positive-controlled, 4-way Crossover Study to Evaluate the Effect of Icosabutate (NST-4016) on the QT/QTc Interval in Healthy Subjects

Objectives:

The primary objective of the study is:

- To evaluate the effects of therapeutic and supratherapeutic concentrations of icosabutate on the Fridericia's corrected QT interval (QTcF) in healthy subjects.

The secondary objectives of the study are:

- To evaluate the effect of therapeutic and supratherapeutic doses of icosabutate on other electrocardiogram (ECG) parameters (heart rate [HR], PR and QRS intervals, and T-wave morphology) in healthy subjects.
- To evaluate the pharmacokinetics (PK) of single doses of 600 mg and 2000 mg icosabutate in healthy subjects.
- To assess the safety and tolerability of single doses of 600 mg and 2000 mg icosabutate in healthy subjects.

Study design:

This will be a Phase 1, single-centre, randomised, double-blind (except for moxifloxacin), placebo- and positive-controlled, 4-way crossover study assessing the ECG effects of therapeutic and supratherapeutic doses of icosabutate in healthy male and female subjects.

Thirty-two subjects will be randomised to a treatment sequence that includes each of the following 4 treatments, administered as a single oral dose:

- therapeutic dose of icosabutate (600 mg)
- supratherapeutic dose of icosabutate (2000 mg)
- moxifloxacin (400 mg)
- placebo

Moxifloxacin will be used as a positive control to determine the assay sensitivity of this study, with an expected peak QT effect ΔΔQTcF of 10 ms to 15 ms.

Each subject will participate in 4 dosing periods (ie, Dosing Periods 1 to 4). Subjects will remain in the Clinical Research Unit (CRU) from Day -1 until Day 18. Doses will be administered in the morning of Days 1, 6, 11, and 16 after an overnight fast of at least 8 hours (not including water). Dose administration in each dosing period will be separated by a wash-out period of at least 5 days. There will be a Follow-up Visit 7 to 10 days after the final dose administration.

Number of subjects:

A total of 32 subjects will be enrolled in order that a minimum of 28 subjects complete the study.

Diagnosis and main criteria for inclusion:

Healthy male and female subjects aged between 18 and 55 years (inclusive) with a body mass index (BMI) between 18.0 and 33.0 kg/m^2 (inclusive).

Investigational products, dose, and mode of administration:

Test products:
600 mg icosabutate (3 × 200 mg capsules; therapeutic dose)
2000 mg icosabutate (10 × 200 mg capsules; supratherapeutic dose)
Administration route: oral.

Reference product and mode of administration:

Reference products:
Placebo capsules matched to icosabutate
400 mg moxifloxacin (positive control)
Administration route: oral

Duration of subject participation in the study:
Planned Screening duration: up to 27 days.
Planned study duration (Screening to Follow-up Visit): approximately 8 weeks.

Endpoints:

Cardiodynamics

The primary endpoint for this study is the placebo-corrected change-from-baseline QTcF (ΔΔQTcF).

The following are the secondary cardiac endpoints for this study:

- change-from-baseline HR, QTcF, PR, and QRS (ie, ΔHR, ΔQTcF, ΔPR, and ΔQRS, respectively)
- placebo-corrected ΔHR, ΔPR, and ΔQRS (ie, ΔΔHR, ΔΔPR, and ΔΔQRS, respectively)
- frequency of T-wave morphology and U-wave presence changes

Continuous 12-lead ECG recordings will be performed from 1 hour predose to approximately 25 hours postdose in each dosing period (ie, Days 1, 6, 11, and 16). All ECG data will be collected using a continuous 12-lead digital recorder, which will be supported by iCardiac Technologies, Inc (an ERT company). At the ECG core laboratory, up to 10 replicate ECGs will be extracted at each of the following timepoints in each dosing period (ie, Days 1, 6, 11, and 16): -45, -30, and -15 minutes predose, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours postdose.

Pharmacokinetics:

Where data allow, the PK parameters for icosabutate will include area under the plasma concentration-time curve from time zero to the time of the last observed concentration (AUC_{0-t}), maximum observed plasma concentration (C_{max}), and time of the maximum observed plasma concentration (T_{max}). Additional PK parameters may be determined, if deemed appropriate. Details of the parameters, and handling procedures, will be described in the Statistical Analysis Plan (SAP). For the purposes of establishing assay sensitivity, moxifloxacin plasma concentrations will be measured to allow concentration-QTc analysis.

Safety:

Adverse events, clinical laboratory evaluations (haematology, clinical chemistry, urinalysis), 12-lead ECGs, vital sign measurements, and physical examinations.

Statistical methods:

Cardiodynamics

The primary analysis will be based on exposure-response modelling of the relationship between icosabutate and ΔΔQTcF with the intent to exclude an effect > 10 ms at clinically relevant icosabutate plasma concentrations.

In addition, the effect of icosabutate on ΔΔQTcF will be evaluated at each postdose timepoint ('by-timepoint' analysis) using the Intersection Union Test. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology and U-wave presence.

Assay sensitivity will be evaluated by exposure-response analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis. If the slope of the exposure-response relationship is statistically significant at 10% level of significance in a 2-sided test and the lower bound of the 2-sided 90% confidence interval (CI) of the predicted QT effect at the observed geometric mean C_{max} following a single dose of 400 mg moxifloxacin is above 5 ms, it will be concluded that assay sensitivity has been demonstrated.

The ECG core laboratory will be responsible for the analysis of the cardiodynamic data.

Pharmacokinetics:

Individual plasma concentrations of icosabutate will be listed and summarised using descriptive statistics.

No formal statistical analysis of PK parameters will be performed.

Safety:

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

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
ΔΔQTcF	placebo-corrected change-from-baseline QTcF
ΔQTcF	change-from-baseline QTcF
AUC_{0-t}	area under the plasma concentration-time curve from time zero to the time of the last observed concentration
ApoC3	apolipoprotein C3
AUC	area under the plasma concentration-time curve
bpm	beats per minute
BMI	body mass index
CI	confidence interval
C_{max}	maximum observed plasma concentration
CRO	Contract Research Organisation
CRU	Clinical Research Unit
CSA	clinical study agreement
EDC	electronic data capture
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic Case Report Form
FSH	follicle-stimulating hormone
HDL-C	high density lipoprotein cholesterol
HR	heart rate
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Council for/Conference on Harmonisation
LDL-C	low density lipoprotein cholesterol
LS	least squares
NSAID	non-steroidal anti-inflammatory drug
OTC	over-the-counter
PK	pharmacokinetic(s)
PPAR	peroxisome proliferator-activated receptor
QTc	QT interval corrected for heart rate
QTcF	Fridericia's corrected QT interval
SD	standard deviation
SE	standard error
T_{max}	time of the maximum observed plasma concentration
TQT	thorough QT
VLDL-TG	very low density lipoprotein triglyceride

1. INTRODUCTION

1.1. Overview

Elevation in triglycerides is associated with dual risks of acute pancreatitis and accelerated atherosclerosis leading to cardiovascular events. Recent genetic studies have strongly suggested a causal relationship between triglyceride levels and ischaemic cardiovascular disease, in addition to other closely interrelated markers such as triglyceride-rich lipoproteins and apolipoprotein C3 (ApoC3). For patients with elevated triglycerides (hypertriglyceridemia) who do not adequately respond to dietary and lifestyle restrictions, there are relatively few classes of drugs available and each is associated with risks that limit their use either alone or in combination.

Icosabutate is a novel, orally administered, highly potent, semi-synthetic fatty acid for the treatment of various dyslipidaemias including severe hypertriglyceridemia, mixed dyslipidaemia, and hypercholesterolemia. In severe hypertriglyceridemia, the primary goal of therapy is to prevent the development of acute pancreatitis by lowering triglyceride levels. Mixed dyslipidaemia is characterised by elevated low density lipoprotein cholesterol (LDL-C) and triglycerides, often accompanied by high density lipoprotein cholesterol (HDL-C) levels. Elevations of triglyceride signal an increased risk of atherosclerotic cardiovascular events even when the LDL-C is well-controlled by statin therapy. The drug substance, icosabutate, is a semi-synthetic derivative of the naturally occurring omega-3 fatty acid eicosapentaenoic acid. The aim of modifying eicosapentaenoic acid is to potentiate the pharmacological effects of naturally occurring omega-3 fatty acids by increasing direct hepatic delivery through the portal vein following absorption, reduce the incorporation into complex lipids, and increase availability for intracellular signalling.

Non-clinical and clinical experience with icosabutate has so far indicated large reductions in both triglycerides and cholesterol, in addition to ApoC3. There are likely multiple contributors to the observed pharmacodynamic effects of icosabutate, a partial peroxisome proliferator-activated receptor (PPAR)α-agonist. Peroxisome proliferator-activated receptor-α is a nuclear receptor expressed in a number of organs including the liver, muscles, heart, and kidneys, and has been recognised as one of the major regulators of glucose and lipid metabolism, playing a role in fatty acid transport, fatty acid binding protein expression, fatty acid-coenzyme A (CoA) synthesis, microsomal, peroxisomal, and mitochondrial oxidation, ketogenesis, fatty acid desaturation, and expression of several apolipoproteins, including ApoC3. However, the lipid changes induced by icosabutate are also partially maintained in PPARα knock-out mice. Icosabutate increases the hepatic clearance of very low density lipoprotein triglyceride (VLDL-TG) without a compensatory increase in hepatic secretion of VLDL-TG and with an improvement in hepatic steatosis. This is consistent with the observed increase in hepatic fatty acid oxidation by icosabutate, which also reduces expression of ApoC3 in animal models and reduces plasma ApoC3 in clinical studies. Expression of ApoC3 is regulated by multiple factors including insulin, glucose, and PPARα, and it plays a key role in regulating triglyceride metabolism (ie, promoting hepatic VLDL-TG secretion and inhibiting peripheral and hepatic clearance of both triglycerides and lipoproteins). Thus, the reductions in ApoC3 conferred by icosabutate may be one of the key drivers of its lipid effects.

1.2. Summary of Non-clinical Pharmacology

The potential cardiovascular effects of icosabutate were evaluated in 4 telemetered male beagle dogs. Each animal received a single oral dose of icosabutate at 0 (corn oil), 100 mg/kg, 550 mg/kg, and 1000 mg/kg in a crossover design with a 6-day interval between doses. Blood pressure, heart rate (HR), and electrocardiogram (ECG) parameters were measured by telemetry at predose and for up to 12 hours postdose and clinical signs were assessed up to 4 hours postdose.

There were no biologically significant changes noted in the blood pressure, HR, ECG intervals or morphology of the ECG waveform at doses of icosabutate up to 1000 mg/kg. Furthermore, no clinical signs were observed at 100 mg/kg. There were 2 events of emesis following 550 mg/kg icosabutate (1 event at 1.25 hours postdose and 1 event at 2.25 hours postdose) and 1 event following 1000 mg/kg icosabutate (1.25 hour postdose).

At the 550-mg/kg and 1000-mg/kg dose levels, mucoid/liquid faeces were observed. No effects on cardiovascular function were observed up to 1000 mg/kg.

Icosabutate was administered at doses of 20 mg/kg/day, 50 mg/kg/day, and 250 mg/kg/day for 52 weeks to male and female dogs in a repeat-dose toxicity study. The reversibility of any changes was assessed over a 4-week recovery to 2 male and 2 female animals from each dose group. During this study, toxicologically significant findings were predominantly confined to the 250-mg/kg/day dose group. Increases in group mean QT, Van de Water corrected QT interval (QTcV) and Fridericia's corrected QT interval (QTcF) were observed in dogs administered 250 mg/kg/day for 52 weeks. In addition, 1 male dog presented with several ventricular escape complexes at 1.5 hours postdose in Week 25. Based on findings in this 52-week toxicity study, the no-observed-adverse-effect-level (NOAEL) in this study was 50 mg/kg/day, which corresponds to respective maximum observed plasma concentration (C_{max}) and area under the plasma concentration-time curve from time zero to the time of the last observed concentration (AUC_{0-t}) values of 23800 ng/mL and 189000 ng·h/mL, respectively, in males and 29500 ng/mL and 168000 ng·h/mL, respectively, in females.

1.3. Summary of Clinical Experience

1.3.1. Safety

As of 24 January 2018, icosabutate has been evaluated in 6 clinical studies, comprising 4 Phase I and 2 Phase II proof-of-concept trials in the target populations.

In the First-in-Human study, icosabutate was administered as single doses between 5 mg and 600 mg, multiples doses of 50 mg to 300 mg twice daily, and 500 mg to 600 mg once daily for up to 28 days. Icosabutate was considered to be safe and well-tolerated, with the overall incidence of adverse events throughout the study being generally low. There were no apparent treatment- or dose-related trends in the number of adverse events reported, or the number of subjects reporting adverse events. There were no other findings of clinical importance in the clinical laboratory evaluations, vital signs, 12-lead ECGs, telemetry, physical examinations, or body weight observed during the study. No dose- or treatment-related trends in ophthalmological assessments were observed following single and multiple oral doses.

In a Phase I high-dose study, single oral doses of 1000 mg, 1400 mg, 2000 mg, and 2800 mg icosabutate were considered to be safe and well tolerated by healthy male and female subjects. All treatment-related adverse events were mild in severity and the majority were gastrointestinal disorders, with incidence tending to increase with increasing dose. There were no treatment- or dose-related findings in the clinical laboratory results, vital signs, 12-lead ECGs, ophthalmological assessments, or physical examinations.

In all Phase I and II studies combined, 265 subjects have received icosabutate; 150 (57%) subjects have reported at least 1 treatment-emergent adverse event, the maximum severity being mild in 114 subjects, moderate in 45 subjects, and severe in 1 subject. Of these 150 subjects, 94 (35%) subjects reported a drug-related treatment-emergent adverse event. No serious adverse events have been reported following the administration of icosabutate.

Three adverse events relating to cardiac or 12-lead ECG findings have been reported in icosabutate-treated patients; all events were non-serious and mild, with 1 event not considered related to study drug by the Investigator, and 2 events being considered possibly related to the study drug by the Investigator.

One event was reported in the First-in-Human Phase I study in healthy and otherwise healthy dyslipidaemic subjects. Following once daily 500 mg icosabutate, 1 subject reported mild palpitations considered by the Investigator to be possibly related to the study drug. The event resolved after 5 minutes.

Two events were reported in the Phase II study in patients with mixed dyslipidaemia. One patient experienced mild sinus bradycardia, which was considered by the Investigator to be not related to study drug. One patient experienced QTc prolongation at the early termination visit, which was mild in severity and considered possibly related to the study drug by the Investigator. No other cardiac-related adverse events were experienced by this subject.

1.3.2. Pharmacokinetics

In all Phase I and II studies combined, icosabutate was rapidly absorbed at all dose levels with a time of the maximum observed plasma concentration (T_{max}) generally within 3 hours postdose at the lower doses, and increasing to 3 to 4 hours postdose at the higher doses. Following chronic dosing the time taken for the concentration of a substance to reduce to half its original value ($t_{½}$) was approximately 5 hours to 11 hours. Icosabutate is excreted both by the kidneys and the liver. Clinical metabolism profiling of clinical plasma samples from 300 mg twice daily in males and 500 mg once daily at Day 14 has been compared with plasma samples from rat and dog after 13-week repeat-dose toxicity studies at 30 mg/kg/day and 50 mg/kg/day, respectively. There were no human unique metabolites, whereas 2 major human disproportionate metabolites were observed with area under the plasma concentration-time curve (AUC) exposure around 10% of total drug-related material. A human radiolabelled absorption, metabolism, and excretion (AME) study showed that the 5 most abundant metabolites in plasma (P1 to P5) accounted for <10% of icosabutate exposure and <5% of the total drug-related exposure. The notable plasma metabolites were characterised as dehydroxylated icosabutate with the loss of $C_{14}H_2O$, two dehydroxylated metabolites of icosabutate hydroxylated icosabutate with the addition of a glucuronide, icosabutate +2H +2O and hydroxylated icosabutate. No icosabutate was detected in urine samples. Only one metabolite (U4) accounted for >10% of the dose administered. The

majority of the remaining components detected in urine accounted for <5% of the total administered dose. As faeces was a relatively minor elimination route, all components accounted for <5% of the dose administered. Two of the metabolites present in faeces were identified as icosabutate +2H +3O and icosabutate +2H+2O.

1.4. Study Rationale

Regulatory guidance (ICH E14 and FDA E14)[1] recommends a thorough QT (TQT) study to be performed on new chemical entities in order to define the ECG effects, with a particular focus on cardiac repolarisation as measured by the QTc duration. This study is being conducted to obtain clear and robust ECG data, using a sufficient sample size, to evaluate the effects of icosabutate on cardiac repolarisation. The study will be conducted in healthy male and female subjects, who are not at risk of arrhythmias.

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 drug(s), 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 associated with icosabutate may be found in the Investigator Brochure (IB).[2]

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objective of the study is:

- To evaluate the effects of therapeutic and supratherapeutic concentrations of icosabutate on the QTcF in healthy subjects.

The secondary objectives of the study are:

- To evaluate the effect of therapeutic and supratherapeutic doses of icosabutate on other ECG parameters (HR, PR and QRS intervals, and T-wave morphology) in healthy subjects.
- To evaluate the pharmacokinetics (PK) of single doses of 600 mg and 2000 mg icosabutate in healthy subjects.
- To assess the safety and tolerability of single doses of 600 mg and 2000 mg icosabutate in healthy subjects.

2.2. Endpoints

2.2.1. Primary Endpoints

The primary endpoint for this study is the placebo-corrected change-from-baseline QTcF (ΔΔQTcF).

2.2.2. Secondary Endpoints

The secondary endpoints for this study are:

- change-from-baseline HR, QTcF, PR, and QRS (ie, ΔHR, ΔQTcF, ΔPR, and ΔQRS, respectively)
- placebo-corrected change-from-baseline HR, PR, and QRS (ie, ΔΔHR, ΔΔPR, and ΔΔQRS, respectively)
- frequency of T-wave morphology and U-wave presence changes

The PK endpoints will include the following plasma PK parameters for icosabutate using non-compartmental methods:

- AUC_{0-t}
- C_{max}
- T_{max}

Additional PK parameters may be determined, if deemed appropriate.

The safety and tolerability endpoints will include the monitoring and reporting of adverse events, and the incidence and magnitude of clinically significant changes from baseline in clinical laboratory values (haematology, clinical chemistry, and urinalysis), vital sign measurements (blood pressure, pulse rate, and oral body temperature), 12-lead safety ECG results, and physical examination findings.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This will be a Phase 1, single-centre, randomised, double-blind (except for moxifloxacin), placebo- and positive-controlled, 4-way crossover study assessing the ECG effects of therapeutic and supratherapeutic doses of icosabutate in healthy male and female subjects.

Thirty-two subjects will be randomised to 1 of 12 treatment sequences (Table 1), with approximately 3 subjects randomised into 8 of the 12 sequences, and 2 subjects randomised into 4 of the 12 sequences, and will receive a single oral dose of each of the following treatments, with a minimum 5-day wash-out between doses:

- therapeutic dose of icosabutate (600 mg)
- supratherapeutic dose of icosabutate (2000 mg)
- moxifloxacin (400 mg)
- placebo

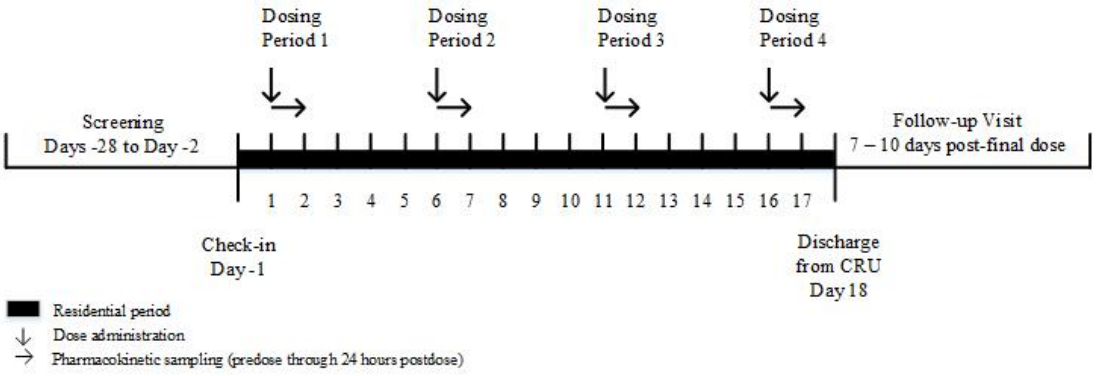
Moxifloxacin will be used as a positive control to determine the assay sensitivity of this study, with an expected peak QT effect ΔΔQTcF of 10 ms to 15 ms.

Table 1: Treatment Sequences

	Dosing Period			
Treatment Sequence	**1 (Day 1)**	**2 (Day 6)**	**3 (Day 11)**	**4 (Day 16)**
1	icosabutate (600 mg)	icosabutate (2000 mg)	placebo	moxifloxacin (400 mg)
2	icosabutate (2000 mg)	moxifloxacin (400 mg)	icosabutate (600 mg)	placebo
3	placebo	icosabutate (600 mg)	moxifloxacin (400 mg)	icosabutate (2000 mg)
4	moxifloxacin (400 mg)	placebo	icosabutate (2000 mg)	icosabutate (600 mg)
5	icosabutate (2000 mg)	placebo	icosabutate (600 mg)	moxifloxacin (400 mg)
6	placebo	moxifloxacin (400 mg)	icosabutate (2000 mg)	icosabutate (600 mg)
7	icosabutate (600 mg)	icosabutate (2000 mg)	moxifloxacin (400 mg)	placebo
8	moxifloxacin (400 mg)	icosabutate (600 mg)	placebo	icosabutate (2000 mg)
9	placebo	icosabutate (600 mg)	icosabutate (2000 mg)	moxifloxacin (400 mg)
10	icosabutate (600 mg)	moxifloxacin (400 mg)	placebo	icosabutate (2000 mg)
11	icosabutate (2000 mg)	placebo	moxifloxacin (400 mg)	icosabutate (600 mg)
12	moxifloxacin (400 mg)	icosabutate (2000 mg)	icosabutate (600 mg)	placebo

Subjects will be screened to assess their eligibility from Day -28 to Day -2. Subjects will be admitted to the Clinical Research Unit (CRU) on Day -1 and remain in the CRU until Day 18. Each subject will participate in 4 dosing periods, with doses administered in the morning of Days 1, 6, 11, and 16 after an overnight fast of at least 8 hours (not including water). Dose administration in each dosing period will be separated by a wash-out period of at least 5 days. There will be a Follow-up Visit 7 to 10 days after the final dose administration. An overview of the study design is shown in Figure 1.

Figure 1: Study Schematic



Abbreviations: CRU = Clinical Research Unit

The total duration of study participation for each subject (from Screening through Follow-up Visit) is anticipated to be approximately 8 weeks.

3.2. Study Start and End of Study Definitions

The start of the study is defined as the date the first enrolled 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. Discussion of Study Design, Including the Choice of Control Groups

This study will be randomised and partially double-blind (double-blind with respect to icosabutate and placebo; open-label with respect to moxifloxacin) and placebo-controlled in order to avoid bias in the collection of data during its conduct. Randomisation eliminates confounding by baseline variables and blinding eliminates confounding by concomitant interventions and biased safety findings, therefore eliminating the possibility that the observed effects of intervention are because of differential use of other treatments or biased expectations regarding safety.

Placebo has been chosen as the control treatment to assess whether any observed effects are treatment-related or simply reflect the study conditions. Moxifloxacin has been chosen as the positive control to demonstrate assay sensitivity. Moxifloxacin has been shown to produce a peak QT interval corrected for heart rate (QTc) prolongation ranging from 10 ms to 15 ms, which is above the threshold of regulatory concern.[1]

The reading and analysis of ECG data will be fully blinded to treatment and timepoint.

A crossover design has been chosen to reduce the variability between treatments; each subject will serve as his/her own control.

Healthy subjects will be enrolled to eliminate variables such as concomitant medications and diseases known to have an effect on ECG parameters. Male and female subjects will be included to eliminate similar known ECG variability effects.

3.4. Selection of Doses in the Study

Icosabutate has been tested at single oral doses between 5 mg and 2800 mg and considered to be safe and well-tolerated (Section 1.3.1). In a Phase II proof-of-concept study, treatment with icosabutate 600 mg resulted in reductions in non-HDL-C compared with placebo that were both statistically significant and clinically relevant. Treatment with icosabutate was well-tolerated and no safety concerns emerged in this study.[2] A total daily dose of 600 mg has been chosen to pursue in subsequent clinical trials and to show any cardiac effects at a therapeutic dose.

The supratherapeutic dose of icosabutate was chosen as it produces the highest exposure in terms of AUC via the oral route.

A 400 mg dose has been for moxifloxacin as this is a standard dose used as a positive control in TQT studies. At this dose it has been consistently demonstrated to increase QTc ranging from 10 ms to 15 ms, which is above the threshold of regulatory concern.[1]

A wash-out period of 5 days is considered sufficient for this study because it spans at least 5 half-lives for icosabutate and moxifloxacin.

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 55 years of age, inclusive, at Screening.
2. Body mass index (BMI) between 18.0 and 33.0 kg/m^2, inclusive, at Screening.
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, Gilbert's syndrome] is not acceptable) at Screening or Check-in (Day -1) 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 Section 6.6. Females of non-childbearing potential are defined as permanently sterile (ie, due to hysterectomy, bilateral salpingectomy, or bilateral oophorectomy) or post-menopausal (defined as at least 12 months post-cessation of menses without an alternative medical cause), confirmed by follicle-stimulating hormone (FSH) assessment (>40 mIU/mL) at Screening, and will not be required to use contraception.
5. Able to comprehend and willing to sign an ICF, to comply with all study activities, 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:

1. An uninterpretable or abnormal ECG at Screening and/or Check-in (Day -1), indicating a second or third degree atrioventricular block, or 1 or more of the following:
    - QRS interval >110 ms;
    - QTcF >430 ms in males and >450 ms in females;
    - PR interval >200 ms;
    - HR <45 beats per minute (bpm);
    - or any rhythm other than sinus rhythm that is interpreted by the Investigator to be clinically significant.
2. History of risk factors for Torsades de Pointes (TdP), including unexplained syncope, known long QT syndrome, heart failure, myocardial infarction, angina, or clinically significant abnormal laboratory assessments including hypokalaemia, hypercalcemia, or hypomagnesemia. Subjects will also be excluded if there is a family history of long QT syndrome or Brugada syndrome.
3. A sustained supine systolic blood pressure >140 mmHg or <90 mmHg, supine diastolic blood pressure >90 mmHg or <50 mmHg, or a resting HR of <45 bpm or >100 bpm at Screening or Check-in (Day -1).
    - Blood pressure may be repeated once in the supine position.
    - The blood pressure abnormality is considered sustained if either the systolic or the diastolic pressure values are outside the above stated limits following 2 assessments.
4. Unstable cardiovascular disease, including recent myocardial infarction or cardiac arrhythmia.
5. 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).
6. History of acquired immunodeficiency syndrome (AIDS) or positive hepatitis panel and/or positive human immunodeficiency virus (HIV) test at Screening (Appendix 2).
7. Significant history of alcoholism or drug/chemical abuse within 2 years prior to Screening, in the opinion of the Investigator.
8. Alcohol consumption of >28 units per week for males and >21 units for females at any time in the 6 months prior to first dose (ie, Day 1). 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.
9. Positive alcohol breath test result, positive urine drug screen (confirmed by repeat), or positive cotinine test at Screening or Check-in (Day -1).

10. Clinically significant illness, including viral syndromes within 3 weeks prior to first dose (ie, Day 1).
11. Female subjects who are pregnant (or planning to become pregnant within 90 days after the final dose administration) or are currently lactating.
12. Participation in a clinical study involving administration of an investigational drug (new chemical entity) in the past 3 months (or 5 half-lives, whichever is longer) prior to first dose (ie, Day 1).
13. Have previously completed or withdrawn from this study or any other study investigating icosabutate, and have previously received the investigational product.
14. Use or intend to use any prescription medications/products (including non-steroidal anti-inflammatory drugs [NSAIDs] or sucralfate and other medications known to prolong the QT/QTc interval), other than hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives, within 14 days (or 5 half-lives, whichever is longer) prior to first dose (ie, Day 1), unless deemed acceptable by the Investigator (or designee).
15. Use or intend to use any non-prescription/over-the-counter (OTC) medications/products including vitamins, minerals, and phytotherapeutic/herbal/plant-derived preparations or omega-3 fish oils within 7 days prior to first dose (ie, Day 1), unless deemed acceptable by the Investigator (or designee).
16. Use or intend to use slow-release medications/products considered to still be active within 14 days (or 5 half-lives, whichever is longer) prior to first dose (ie, Day 1), unless deemed acceptable by the Investigator (or designee).
17. 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 first dose (ie, Day 1), unless deemed acceptable by the Investigator (or designee).
18. Consumption of food and beverages containing poppy seeds, Seville oranges, grapefruit, or grapefruit juice, within 7 days prior to Check-in (Day -1; Section 6.2).
19. Consumption of caffeine- or xanthine-containing products or alcohol within 72 hours prior to Check-in (Day -1; Section 6.2).
20. Use of tobacco- or nicotine-containing products (eg, cigarettes, cigars, chewing tobacco, snuff, or nicotine-replacement products) within 30 days prior to first dose (Day 1).
21. Strenuous activity (eg, sports), or intent to participate in strenuous activity from 4 days before Check-in (Day -1) and throughout the study (until after the final Follow-up Visit).
22. Donation of more than 500 mL blood (or significant blood loss) from 3 months prior to Screening, plasma from 2 weeks prior to Screening, or platelets from 6 weeks prior to Screening.
23. Receipt of blood products within 2 months prior to Check-in (Day -1).

24. History of significant hypersensitivity, intolerance, or allergy to any drug compound (including moxifloxacin), food, or other substance, unless approved by the Investigator (or designee).
25. Any other medical, psychological, or social condition that, in the opinion of the Investigator or the medical monitor, would prevent the subject from fully participating in the study, would represent a concern for study compliance, or would constitute a safety concern to the subject.
26. Poor peripheral venous access.
27. Unable to or may have difficulties swallowing a potentially large number of capsules.
28. Study site staff or immediate family members of study site staff either directly or indirectly involved with the present study.
29. Subjects who, in the opinion of the Investigator (or designee), should not participate in this study.

Subjects may previously have been screened on a generic basis to determine their eligibility for inclusion in Phase I clinical studies conducted at the CRU. 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 CRU on Day -1.

4.3. Subject Number and Identification

Subjects will have a unique identification number used at Screening. Subjects will be assigned a subject number immediately after the first dose occasion, at the time of their randomisation. Assignment of subject numbers will be in ascending order and no numbers will be omitted (eg, Subjects 101, 102, 103, etc). Replacement subjects (Section 4.4) 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 subject number only on all study documentation. A list identifying the subjects by subject number will be kept in the Site Master File (SMF).

4.4. 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)
- 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, 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, efforts will be made to perform all follow-up assessments, if possible (Appendix 5). 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 CRU. 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 adverse events or until the unresolved adverse events are judged by the Investigator (or designee) to have stabilised.

Subjects who are withdrawn for reasons not related to study drug may be replaced following discussion between the Investigator and the Sponsor. Subjects withdrawn as a result of adverse events thought to be related to the study drug will generally not be replaced.

4.5. 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:

- adverse events 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 adverse events (this may also apply to adverse events defined at Check-in [Day -1] 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 study drugs (200 mg icosabutate capsules in Self-Micro Emulsifying Drug Delivery System [SMEDDs] and placebo capsules) will be supplied by the Sponsor (or designee), along with the batch/lot number(s) and Certificate(s) of Analysis. A Certificate of Release authorised by a Qualified Person in the European Union will also be issued for the study drugs. The study drugs will be provided in high-density polyethylene (HDPE) bottles and stored according to the instructions on the label.

All study drugs 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 study drugs will be transferred from bulk supplies into the subject's dose container by qualified clinical staff.

Moxifloxacin tablets (400 mg) are commercially available in the EU and will be sourced by the CRU. The moxifloxacin tablets will be stored in accordance with the requirements detailed in the package insert. The moxifloxacin tablets will be subject to accountability procedures and unused supplies will be destroyed at the end of the study.

5.2. Study Treatment Administration

Subjects will be dosed at the time indicated in the Schedule of Assessments (Appendix 5).

Each dose of icosabutate, placebo, and moxifloxacin will be administered orally with approximately 240 mL of room temperature water. If the capsules cannot be swallowed at the same time, drug administration may be divided, but dosing should be completed within 2 minutes of the scheduled time. Additional water up to a maximum of 50 mL may be administered if needed by the subject. All doses will be administered on the mornings of Days 1, 6, 11, and 16 following an overnight fast (not including water) of at least 8 hours, and will be followed by a fast (not including water) for at least 4 hours postdose. Except as part of dose administration, subjects will restrict their consumption of water from 1 hour predose until 2 hours postdose; water will be freely available at all other times.

The number of capsules to be administered on each respective dosing occasion are presented in Table 2.

Table 2: Number of Capsules to be Administered on Each Respective Dosing Occasion

	Number of Capsules		
	Icosabutate[a]	**Placebo[b]**	**Moxifloxacin**
600 mg icosabutate (therapeutic dose)[c]	3	7	NA
2000 mg icosabutate (supratherapeutic dose)[c]	10	NA	NA
400 mg moxifloxacin (positive control)[c]	NA	NA	1
Placebo[c]	NA	10	NA

Abbreviations: NA = not applicable.
a. Supplied as 200 mg icosabutate in Self-Micro Emulsifying Drug Delivery System (SMEDDS).
b. Placebo capsules matched to icosabutate.
c. Subjects will be randomised to 1 of 12 treatment sequences and will receive a single oral dose of each of the treatments, with a minimum 5-day wash-out between doses. To maintain the blind, with the exception of dosing with moxifloxacin, the same number of capsules will be given on each dosing occasion.

Subjects will be administered the study drugs 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 adverse event(s) and/or study procedures. A hand-and-mouth check will be performed to verify that the dose administered was swallowed.

5.3. Randomisation

The randomisation code will be produced by the statistics department at Covance using a computer-generated pseudo-random permutation procedure. Subjects will be randomised to 1 of 12 treatment sequences (Table 1).

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

5.4. Blinding

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

- The placebo capsules will be identical in appearance to icosabutate.
- The Investigator and other members of staff involved with the study will remain blinded to the treatment randomisation code during the assembly procedure.
- With the exception of dosing with moxifloxacin, the same number of capsules will be given on each dosing occasion (Table 2).

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 (in the event of possibly treatment-related serious adverse events or severe adverse events), 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.
- At each dosing occasion, a predose and postdose inventory 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 medications/products (including NSAIDs or sucralfate and other medications known to prolong the QT/QTc interval) from 14 days (or 5 half-lives, whichever is longer) or non-prescription/OTC medications/products including vitamins, minerals, and phytotherapeutic/herbal/plant-derived preparations or omega-3 fish oil from 7 days prior to first dose (ie, Day 1) and 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 in a medical emergency. 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 (not including water; at least 8 hours) before collection of blood samples for clinical laboratory evaluations.

The subjects will be fasted overnight (not including water; at least 8 hours) prior to dosing and will 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. Other than these fluid restrictions, water will be freely available at all times.

Foods and beverages containing poppy seeds, Seville oranges, grapefruit, or grapefruit juice will not be allowed from 7 days prior to Check-in (Day -1) until the Follow-up Visit.

Caffeine- or xanthine-containing foods and beverages will not be allowed from 72 hours prior to Check-in (Day -1) until discharge from the CRU (Day 18).

Consumption of alcohol will not be permitted from 72 hours prior to Check-in (Day -1) until discharge from the CRU (Day 18). Up to 2 units/day of alcohol are permitted from discharge until 36 hours before the Follow-up Visit.

6.3. Smoking

Subjects will not be permitted to use tobacco- or nicotine-containing products within 30 days prior to first dose (ie, Day 1) until the Follow-up Visit.

6.4. Exercise

Subjects are required to refrain from strenuous exercise from 4 days before Check-in (Day -1) until after the final 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.

6.6. Contraception

Female subjects who are of non-childbearing potential will not be required to use contraception. Females of non-childbearing potential are defined as permanently sterile (ie, due to hysterectomy, bilateral salpingectomy, or bilateral oophorectomy), confirmed by history, or post-menopausal (defined as at least 12 months post-cessation of menses without an alternative medical cause). Post-menopausal status will be confirmed with a screening serum FSH level >40 mIU/mL.

Female subjects of childbearing potential must be willing to use a highly effective method of birth control (ie, contraceptive measure with a failure rate of <1% per year) in conjunction with male barrier contraception (ie, male condom with spermicide) from the time of signing the ICF until 90 days after the last dose of study drug. Highly effective methods of contraception include:

- established use of oral, implantable, transdermal, or injectable hormonal method of contraception associated with inhibition of ovulation
- hormonal or non-hormonal intrauterine device (IUD; eg, Mirena®).
- bilateral tubal ligation or occlusion (performed at least 90 days prior to the Screening Visit)
- male sterilisation (performed at least 90 days prior to the Screening Visit), with verbal confirmation of surgical success (for female subjects on the study, the vasectomised male partner should be the sole partner for that subject)

Female subjects of childbearing potential should refrain from donation of ova from Check-in (Day -1) until 90 days after the last dose of study drug.

Male subjects 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 used by their female partners from Check-in (Day -1) until 90 days after the last dose of study drug. In addition to the list of highly effective contraception methods above, other acceptable methods of contraception for the female partner include:

- established use of progesterone only oral contraception, where inhibition of ovulation is not the primary mode of action
- diaphragm, cap, or sponge in conjunction with spermicide.

For male subjects, 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 last dose of study drug. Male subjects are required to refrain from donation of sperm from Check-in (Day -1) until 90 days after the last dose of study drug.

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, post-ovulation 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.

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 order of priority for scheduling procedures around a timepoint is (in descending order of priority):

- dosing
- ECG extraction
- blood samples
- any other procedures (safety ECGs will be scheduled before vital sign measurements)

7.1. Pharmacokinetic Assessments

7.1.1. Sample Collection and Processing

Blood samples (approximately 1 × 2.0 mL) will be collected by venepuncture or cannulation at the times indicated in the Schedule of Assessments (Appendix 5). Furthermore, up to 3 additional blood samples may be taken from each subject per dosing period, 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 Trial Master File (TMF). Samples taken from subjects following the administration of placebo will not be analysed.

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 icosabutate and moxifloxacin will be determined using validated analytical procedures. Specifics of the analytical methods will be provided in separate document(s).

7.2. Cardiodynamic (Continuous) 12-lead Electrocardiogram Recordings

7.2.1. Electrocardiogram Collection

Electrocardiograms for the assessment of QT/QTc will be recorded continuously, which will obtain ECGs prior to, and for approximately 25 hours after, administration of study drug in each treatment period. The cardiodynamic assessments will be performed through 12-lead ECGs extracted from continuous recordings at pre-specified timepoints, paired with PK samples, at the times indicated in the Schedule of Assessments (Appendix 5).

To ensure quality ECG extractions, subjects will be resting (supine) for at least 10 minutes before and 5 minutes after each ECG extraction window. The ECGs will be extracted from continuous 24-hour ECG recording during an extraction window of 5 minutes after the nominal timepoint. Environmental distraction (eg, television, playing games, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording.

7.2.2. QTc Analysis

Continuous 12-lead ECG recordings will be performed from 1 hour predose to approximately 25 hours postdose in each dosing period (ie, Days 1, 6, 11, and 16).

The 12-lead ECGs to be used in the analyses will be selected by pre-determined timepoints as defined in the Schedule of Assessments (Appendix 5). At the ECG core laboratory, up to 10 replicate ECGs will be extracted at each of the following timepoints in each dosing period (ie, Days 1, 6, 11, and 16): -45, -30, and -15 minutes predose, and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours postdose.

The following principles will be followed in the ECG core laboratory when data are analysed:

- The ECG analysts are blinded to the subject, visit, and treatment allocation.
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analysed by the same reader.
- The primary analysis lead is lead II. If lead II is not analysable, then primary lead of analysis will be changed to another lead for the entire subject data set.

A brief description of the ECG analysis methods utilised by iCardiac's core laboratory are presented in Sections 7.2.3 and 7.2.4.

7.2.3. 12-lead Electrocardiogram Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from continuous Holter recordings using the 'TQT Plus method'. This method enables extraction of ECGs with the

lowest HR variability and noise within the protocol-specified extraction time window (eg, the HR and QT changes from beat-to-beat in the range of <10%). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute 'ECG window' (typically, the last 5 minutes of the 15 minute period when the subject is maintained in a supine or semi-recumbent quiet position).

7.2.4. High-precision QT Analysis

High-precision QT analysis will be performed on all analysable (non-artefact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify 'high' and 'low' confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely)
- RR values exceeding or below certain thresholds (biologically unlikely)
- Rapid changes in QT, QTc, or RR from beat-to-beat

Measurement of all primary ECG parameters (QT, QTc, and RR) in all recorded beats of all replicates that are deemed 'high confidence' is performed using COMPAS software. All 'low confidence' beats are reviewed manually and adjudicated using pass-fail criteria. The final quality control assessment is performed by a cardiologist. The beats found acceptable by manual review will be included in the analysis. The median QT, QTc, and RR value from each extracted replicate will be calculated, and the mean of all available medications from a nominal timepoint will be used as the subject's reportable value at that timepoint.

Categorical T-wave morphology analysis and the measurement of PR and QRS intervals will be performed manually in 3 of the 10 ECG replicates at each extraction timepoint. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) will be electronically marked. The T-wave morphology categories are presented in Table 3.

Table 3: T-wave Morphology Categories

Category	Description
Normal T-wave	Any T-wave not meeting any criterion below
Flat T-wave	T amplitude <1 mm (either positive or negative) including flat isoelectric line
Notched T-wave	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave
Biphasic T-wave	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive and negative/positive polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notch(es)
Notched T-wave(-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave

In addition to the T-wave categorical analysis, the presence of abnormal U-waves will be noted.

The ECG extractions will be time-matched to the PK samples; however, these will be obtained prior to the actual PK sampling time in order to avoid changes in autonomic tone

associated with the psychological aspects of blood collection, in addition to the reduction in blood volume subsequent to blood collection.

7.3. Safety and Tolerability Assessments

7.3.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting serious adverse events 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 adverse events occurring at any other time during the study.

Any adverse events 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 drug.

Adverse events recorded during the course of the study will be followed up, where possible, until resolution. This will be completed at the Investigator's (or designee's) discretion.

7.3.2. 12-lead Safety Electrocardiogram

Cardiac safety will be assessed by Investigator evaluation of standard 12-lead ECGs measured using ECG recorders at the times indicated in the Schedule of Assessments (Appendix 5). Resting 12-lead ECGs will be recorded after the subjects has been supine and at rest for at least 5 minutes. Single 12-lead ECGs will be repeated once if either of the following criteria apply:

- QTcF value >450 ms for females or >430 ms for males
- 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. The median value will be used as the baseline value in the data analysis. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges.

7.3.3. Vital Signs

Supine blood pressure, supine pulse rate, and oral body temperature will be assessed at the times indicated in the Schedule of Assessments (Appendix 5), and repeated once if outside the relevant clinical reference ranges. 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.

Subjects must be supine for no less than 5 minutes before blood pressure and pulse rate measurements.

7.3.4. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations (including clinical chemistry, haematology, urinalysis, and serology) at the times indicated in the Schedule of Assessments (Appendix 5). Clinical laboratory evaluations are listed in Appendix 2. Additional clinical laboratory evaluations will 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 drugs of abuse screen and cotinine test, and will undergo an alcohol breath test at the times indicated in the Schedule of Assessments (Appendix 5). For all female subjects, a pregnancy test and FSH will be performed at the times indicated in the Schedule of Assessments (Appendix 5).

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

7.3.5. Physical Examination

A full physical examination or abbreviated physical examination will be performed at the timepoints specified in the Schedule of Assessments (Appendix 5).

7.3.6. Body Weight and Height

Body weight (in underclothes) and height (for calculation of BMI) will be recorded at the times indicated in the Schedule of Assessments (Appendix 5).

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

A total of 32 subjects will be enrolled in order that a minimum of 28 subjects complete the study. Based on experience from the IQ-CSRC study[3] simulations to evaluate the power of small studies with exposure-response analysis[4], and the observations from 25 recent TQT studies using exposure-response analysis, a sample size of 28 will provide more than 95% power to exclude that icosabutate causes more than a 10-ms QTc effect at clinically relevant plasma levels, as shown by the upper bound of the 2-sided 90% confidence interval (CI) of the model predicted QTc effect (ΔΔQTcF) at the observed geometrical mean C_{max} of icosabutate in the study.

In addition to the evaluation through modelling and simulation, the sample size can be estimated approximately using a simple paired t-test for equivalence. Under the assumption that the QTc effect is 3 ms for icosabutate and 0 ms for placebo with a common standard deviation (SD) of change-from-baseline QTcF (ΔQTcF) of 8 ms for each treatment, and that

'No Effect' will be concluded if the 90% CI of ΔΔQTcF is lower than 10 ms, 28 subjects will provide >95% power with a one-sided alpha of 5% in paired t-test. This estimation was performed using the paired t-test for equivalence of Means in R Version 3.2.5.

8.2. Sample Size Considerations for Assay Sensitivity

To demonstrate assay sensitivity with exposure-response analysis, it has to be shown that the ΔΔQTcF of a single dose of 400 mg moxifloxacin exceeds 5 ms (ie, the lower bound of the 2-sided 90% CI of the predicted QTc effect [ΔΔQTcF] should exceed 5 ms). In a similarly designed recent crossover study with 24 healthy subjects, the standard error (SE) for the prediction of the QT effect of moxifloxacin based on the exposure-response analysis was 1.24 ms. The within-subject SD of ΔQTcF in the referred study was 5.4 ms. If the effect of moxifloxacin is assumed to be 10 ms, the SE of 1.24 ms corresponds to an effect size of $\left({}^{(10-5)}\!/_{(1.24\times\sqrt{24})}\right) = 0.82$, where the effect size is the effect assumed under the alternative hypothesis divided by the SD of the test variable. In a one-sample t-test situation with a sample size of 24 subjects, this effect size results in a power of 98.8%. This value should be compared with the effect size of 0.57 required to guarantee a power of at least 90% in a one-sample t-test situation with a sample size of 28 subjects. Based on this calculation, a power of at least 90% will be obtained as long as the variability of ΔQTcF, as measured by its SD, does not exceed 7.8 ms (ie, 144% of the 5.4 ms observed in the referred study).

8.3. Analysis Populations

8.3.1. Safety Population

The Safety Population will include all subjects enrolled in the study who receive at least 1 dose of study drug (icosabutate, moxifloxacin, or placebo).

8.3.2. Pharmacokinetic Population

The Pharmacokinetic Population will include all subjects who received at least 1 dose of icosabutate or moxifloxacin and have evaluable PK data. Subjects will be excluded from the PK summary statistics and statistical analysis if a subject has an adverse event of vomiting that occurs at or before 2 times median time to maximum concentration.

8.3.3. QT/QTc Population

The QT/QTc Population will include all subjects in the Safety Population with measurements at baseline as well as on-treatment with at least 1 postdose timepoint with a valid change-from-baseline QTcF (ΔQTcF) value. The QT/QTc population will be used for the analysis of ECG parameters.

8.3.4. Pharmacokinetic/QTc Population

The Pharmacokinetic/QTc Population will include all subjects who are in both the Pharmacokinetic and QT/QTc Populations with at least 1 pair of postdose PK and QTcF data from the same timepoint. The Pharmacokinetic/QTc population will be used for the exposure-response analysis and will be defined for icosabutate and for moxifloxacin.

8.4. Statistical Analyses of Cardiodynamic (Continuous) 12-lead Electrocardiogram Data

The primary analysis will be based on exposure-response modelling of the relationship between icosabutate and ΔΔQTcF, with the intent to exclude an effect >10 ms at clinically relevant icosabutate plasma concentrations.

In addition, the effect of icosabutate on ΔΔQTcF will be evaluated at each postdose timepoint ('by-timepoint' analysis) using the Intersection Union Test. An analysis of categorical outliers will be performed for changes in HR, PR, QRS, QTcF, T-wave morphology, and U-wave presence.

Assay sensitivity will be evaluated by exposure-response analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis. If the slope of the exposure-response relationship is statistically significant at 10% level of significance in a 2-sided test and the lower bound of the 2-sided 90% CI of the predicted QT effect at the observed geometric mean C_{max} following a single dose of 400 mg moxifloxacin is above 5 ms, it will be concluded that assay sensitivity has been demonstrated.

8.4.1. Baseline

For all 12-lead ECG parameters from each dosing period, baseline will be the average of the derived ECG intervals from the 3 ECG timepoints prior to dosing (ie, -45, -30, and -15 minutes) at each dosing period (Days 1, 6, 11, and 16).

8.4.2. Exposure-response Analysis

The exposure-response analysis will be based on ΔΔQTcF; ie, for the placebo adjustment, the individual ΔQTcF for placebo calculated at a specific timepoint is subtracted from ΔQTcF for the same subject on icosabutate at the same timepoint to generate ΔΔQTcF. The relationship between icosabutate plasma concentration and ΔΔQTcF will be investigated by linear mixed-effects modelling. The following 3 linear models will be considered:

- Model 1; a linear model with an intercept
- Model 2; a linear model with mean intercept fixed to 0 (with variability)
- Model 3; a linear model with no intercept

Time-matched concentration of icosabutate will be included in the model as a covariate and subject as a random effect for both intercept and slope, when applicable. The model that fits the data best (ie, has the smallest Akaike Information Criterion and model-predicted CI similar to the observed CIs) will be used for predicting population average ΔΔQTcF and its corresponding 2-sided 90% CI at the observed mean C_{max}. If the upper bound of the 2-sided 90% CI of the predicted effect at this plasma level is below 10 ms, it will be concluded that icosabutate does not cause clinically relevant QTc prolongation.

The plot of the observed median-quantile icosabutate concentrations and associated mean ΔΔQTcF interval (90% CI) together with the regression line presenting the predicted ΔΔQTcF interval (90% CI)[5] will be used to evaluate the adequacy of the model fit of the primary model to the assumption of linearity and the impact on quantifying the

exposure-response. For evaluation of the HR-QTc interval, scatter plot of QTcF and RR intervals by treatment with regression line will be also given. Additional exploratory analyses (via graphical displays and/or model fitting) will include accounting for a delayed effect (hysteresis) and the justification for the choice of pharmacodynamic model (linear versus nonlinear) as follows.

8.4.2.1. Investigation of Hysteresis

Hysteresis will be assessed based on joint graphical displays of the least squares (LS) mean difference between ΔQTcF under icosabutate and under placebo (ΔΔQTcF) for each postdose timepoint and the mean concentrations of icosabutate at the same timepoints. In addition, hysteresis plots will be given for mean ΔΔQTcF and each of the mean concentrations. If a QT effect (ΔΔQTcF) >10 ms cannot be excluded from the by-timepoint analysis and if a delay between peak ΔΔQTcF and peak plasma concentration in the plot (ΔΔQTcF versus icosabutate) of more than 1 hour is observed, other exposure-response models, such as a model with an effect compartment, may be explored. With the provision stated above, hysteresis will be assumed if the curve shows a counterclockwise loop.

8.4.2.2. Appropriateness of a Linear Model

To assess the appropriateness of a linear model, normal Q-Q plots for the residuals and plots of weighted residuals versus concentration and versus fitted values will be produced. The scatter plot of residuals versus concentration by LOESS fitting (ie, locally weighted scatter plot smoothing)[6] will also be produced with an optimal smoothing parameter selected by the Akaike Information Criterion with a correction (AICC).[7] In addition, a model with both the original term and a quadratic term in concentration will be fitted and the quadratic term will be tested on the 2-sided 5% level. If there is an indication that a linear model is inappropriate, additional models will be fitted; in particular:

- a maximum response (E_{max}) model
- a log-transformation model where the plasma concentration ‘C’ is replaced by ln/(C/C0), where C0 is the limit of quantification of the assay used to determine C, and all values below C0 are replaced by C0 (ie, log [C0/C0]=0)

The exposure-response analysis will be repeated for the model found to best accommodate the nonlinearity detected.

8.4.3. Assay Sensitivity

To determine assay sensitivity, the exposure-response (ΔΔQTcF) of 400 mg oral moxifloxacin will be utilised. If the slope of the moxifloxacin plasma concentration/ΔΔQTcF relationship is statistically significant at the 10% level in a 2-sided test and the lower bound of the 2-sided 90% CI of the predicted QT effect at the geometric mean C_{max} following a single dose of 400 mg moxifloxacin is above 5 ms, it will be concluded that assay sensitivity has been demonstrated.

8.4.4. By-timepoint Analysis (Secondary Analysis)

The 'by-timepoint analysis' for QTcF will be based on a linear mixed-effects model with ΔQTcF as the dependent variable, period, sequence, time (ie, timepoint will be categorical), treatment (icosabutate, moxifloxacin, and placebo), and time-by-treatment interaction as fixed effects, and baseline QTcF as a covariate. An unstructured covariance matrix will be specified for the repeated measures at postdose timepoints for subject within dosing period. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as autoregressive and compound symmetry will be considered. If the fixed effects for period and/or sequence should prove to be non-significant (ie, if the p-value >0.1), these effects may be removed from the model and the analysis will be repeated without those covariates. Subjects dosed with placebo will be analysed as a pooled group. From this analysis, the LS means and 2-sided 90 % CI will be calculated for the contrast 'icosabutate versus placebo' at each dose of icosabutate and each postdose timepoint on Days 1, 6, 11, and 16 in addition to each predose timepoint on Days 6, 11, and 16, separately.

For HR, PR, and QRS interval, the analysis will be based on the change-from-baseline postdose (ΔHR, ΔPR, and ΔQRS, respectively). The same (by-timepoint analysis) model will be used as described for QTcF. The LS mean, SE and 2-sided 90% CI from the statistical modelling for both change-from-baseline and placebo-corrected change-from-baseline values will be listed in the tables and graphically displayed.

8.4.5. Categorical Analysis

The analysis results for categorical outliers, T-wave morphology, and U-wave presence will be summarised in frequency tables with counts percentages for both number of subjects and number of timepoints. For categorical outliers, the number (percentage) of subjects in addition to timepoints with increases in absolute QTcF interval values >450 ms and ≤480 ms, >480 ms and ≤500 ms, and >500 ms, and changes from predose >30 ms and ≤60 ms, and >60 ms; increase in PR interval from predose >25% to a PR >200 ms; increase in QRS interval from predose >25% to a QRS >120 ms; decrease in HR from predose >25% to a HR <50 bpm; and increase in HR from predose >25% to a HR >100 bpm will be determined.

For T-wave morphology and U-wave presence, the analysis will be focused on change from baseline, ie, treatment-emergent changes (across all timepoints).

8.5. Pharmacokinetic Analyses

Non-compartmental PK analysis will be performed on individual plasma concentration data, using commercial software such as Phoenix® WinNonlin®. Plasma concentrations of icosabutate and PK parameters will be listed and summarised using descriptive statistics.

For each subject, the following PK parameters will be calculated for icosabutate, where possible:

- AUC_{0-t}: area under the plasma concentration-time curve from time zero to the time of the last observed concentration
- C_{max}: maximum observed plasma concentration
- T_{max}: time of the maximum observed plasma concentration

Additional PK parameters may be determined, if deemed appropriate. Details of the parameters, and handling procedures, will be described in the Statistical Analysis Plan (SAP).

For the purposes of establishing assay sensitivity, moxifloxacin plasma concentrations will be measured to allow concentration QTc analysis.

Plasma concentrations and PK parameters will be summarised by treatment using descriptive statistics. No formal statistical analysis of PK parameters will be performed.

8.6. Safety Analysis

Safety will be evaluated in terms of reported adverse events and other clinical observations, clinical laboratory test results (haematology, clinical chemistry, and urinalysis), vital sign measurements (blood pressure, pulse rate, and oral body temperature), 12-lead safety ECG results, and physical examination findings.

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

8.7. Interim Analysis

No interim analyses are planned for this study.

9. REFERENCES

1. Food and Drug Administration. Guidance for Industry: E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-antiarrhythmic Drugs. Food and Drug Administration; 2005.

2. NorthSea Therapeutics. NST-4016 - Investigator's Brochure. Version 7, 24 January 2018.

3. Darpo B, Benson C, Dota C, Ferber G, Garnett C, Green CL, et al. Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. Clin Pharmacol Ther. 2015 Apr;97(4):326–35.

4. Ferber G, Zhou M, Darpo B. Detection of QTc effects in small studies--implications for replacing the thorough QT study. Ann Noninvasive Electrocardiol Off J Int Soc Holter Noninvasive Electrocardiol Inc. 2015 Jul;20(4):368–77.

5. Tornøe CW, Garnett CE, Wang Y, Florian J, Li M, Gobburu JV. Creation of a knowledge management system for QT analyses. J Clin Pharmacol. 2011 Jul;51(7):1035–42.

6. S. Cleveland W, J. Devlin S. Locally Weighted Regression: An Approach to Regression Analysis by Local Fitting. J Am Stat Assoc. 1988 Sep 1;83:569–610.

7. Hurvich CM, Simonoff JS, Tsai C-L. Smoothing parameter selection in nonparametric regression using an improved Akaike information criterion. J R Stat Soc Ser B Stat Methodol. 2002 Jan 6;60(2):271–93.

10. APPENDICES

APPENDIX 1: Adverse Event Reporting

Definitions

An adverse event 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 adverse event can therefore be any unfavourable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not related to the study drug.

Assessment of Severity

The Investigator will be asked to provide an assessment of the severity of the adverse event 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 significant affects clinical status, or may require intensive therapeutic intervention.

Relationship to Study Treatment

The Investigator will make a determination of the relationship of the adverse event to the study drug using a 2-category system according to the following guidelines:

- **Not Related**: The adverse event is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Related**: The adverse event 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 adverse events. Any subject who has an ongoing adverse event that is related to the study drug (icosabutate, placebo, or moxifloxacin) or study procedures at the Follow-up Visit will be followed up, where possible, until resolution. This will be completed at the Investigator's (or designee's) discretion. Any subject who has an ongoing adverse event that is not related to the study drug 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 a study drug (ie, where a causal relationship between a study drug and an adverse event 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 humans 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 investigational medicinal product [IMP]).

Serious Adverse Events

A serious adverse event 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 serious adverse events when, based upon appropriate medical judgment, 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.

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

An adverse event 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 Clinical Research Unit (CRU). When in doubt as to whether hospitalisation occurred or was necessary, the adverse event should be considered as serious.

Hospitalisation for elective surgery or routine clinical procedures, which are not the result of an adverse event, need not be considered adverse events 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 adverse event and either 'serious' or 'non-serious' attributed according to the usual criteria.

Serious Adverse Event Reporting

The Sponsor, NorthSea Therapeutics BV, DC Naarden, The Netherlands are responsible for coordinating the reporting of serious adverse events in accordance with the European Directive 2001/20/EC.

The Investigator will complete a serious adverse event report form and forward it by email or fax to Bionical Emas immediately (within 24 hours) upon becoming aware of a serious adverse event.

Email: drug.safety@bionical-emas.com

Or

Fax: +44 (0)1462 600456

The responsibilities of Bionical Emas include the following:

- Prepare a serious adverse event reporting plan prior to the start of the study. Where this plan differs from the applicable CRU standard operating procedure on serious adverse event reporting, the adverse event reporting plan will always take precedence.
- Write serious adverse events case narratives for entry into the PV-works database.
- Produce appropriate reports of all Suspected Unexpected Serious Adverse Reactions and forward to the Ethics Committee, Medicines and Healthcare Products Regulatory Agency, and Principal Investigator 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

Female subjects of childbearing potential will be instructed to use adequate contraceptive precautions until 90 days after the last dose of study drug. To ensure subjects' safety, each pregnancy in a subject who has received study drug must be reported using the appropriate

pregnancy form and forward it by email or fax to Bionical Emas (contact details below) within 24 hours of learning of its occurrence (either from the result of a urine pregnancy test or the verbal information). The pregnancy outcomes, including spontaneous or voluntary termination, details of the birth, and the presence/absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications will be followed. Any serious adverse events experienced during pregnancy should also be reported.

Female subjects who are of non-childbearing potential, subjects who practice true abstinence, and subjects who are exclusively in same-sex relationships will not be required to use contraception.

Email: drug.safety@bionical-emas.com

Or

Fax: +44 (0)1462 600456

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 protein Urea Uric acid	Haematocrit Haemoglobin Mean cell haemoglobin (MCH) Mean cell haemoglobin concentration (MCHC) Mean cell volume (MCV) 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:**[b]	**Hormone panel - females only:**
Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency (HIV-1 and HIV-2) antibodies	Including but not limited to: Amphetamines/methamphetamines Barbiturates Benzodiazepines Cocaine (metabolite) Methadone Phencyclidine Opiates Tetrahydrocannabinol/cannabinoids Tricyclic antidepressants Alcohol breath test Cotinine	Follicle-stimulating hormone (FSH)[c] Serum pregnancy test (human chorionic gonadotropin)[c] Urine pregnancy test[d,e]

a. Only analysed at Screening.
b. Only analysed at Screening and Check-in (Day -1).
c. Performed at Screening for all females.
d. Performed at Check-in (Day -1) and Follow-up at 7 to 10 days after the final dose administration.
e. 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)
Serology	3.5	1	3.5
Clinical laboratory evaluations (clinical chemistry and haematology)[a]	7.5	6	45.0
Pharmacokinetic samples	2.0	64[b]	128.0
Total:			176.5

a. Includes pregnancy and follicle-stimulating hormone (FSH) test for all female subjects; a pregnancy test is performed in serum at Screening.
b. Includes an additional 3 blood samples per dosing period (Section 7.1.1).

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 255 mL.

APPENDIX 4: 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 Brochure (IB), and other relevant documents must be submitted to an Ethics Committee (EC) by the Investigator and reviewed and approved by the 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 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 EC annually or more frequently in accordance with the requirements, policies, and procedures established by the EC.
- Notifying the EC of serious adverse events or other significant safety findings as required by 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 EC, European regulation 536/2014 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 drugs, 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 Clinical Research Unit (CRU) personnel, subjects will sign 2 copies of the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. One copy will be given to the subject, and the other will be maintained in the subject's records.

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 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 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 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, EC review, and regulatory agency inspections and provide direct access to source data documents.
- Covance is responsible for the data management of this study including quality checking of the data. Pre-defined, agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. 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 Good Clinical Practice (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 Code of Federal Regulations (CFR) Part A1-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 Covance electronically, will be integrated with the subject's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each subject who signs an ICF and 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

If on completion of the study the data warrant publication, the Investigator may publish the results in recognised (refereed) scientific journals subject to the provisions of the clinical study agreement (CSA). Unless otherwise specified in the CSA, the following process shall occur:

If the Investigator expects to participate in the publication of data generated from this site, the institution and Investigator shall submit reports, abstracts, manuscripts, and or other presentation materials to the Sponsor for review before submission for publication or presentation. The Sponsor shall have 60 days to respond with any requested revisions, including without limitation, the deletion of confidential information. The Investigator shall act in good faith upon requested revisions, except the Investigator shall delete any confidential information from such proposed publications. The Investigator shall delay submission of such publication or presentation materials for up to an additional 90 days in order to have a patent application(s) filed.

APPENDIX 5: Schedule of Assessments

Table 4: Schedule of Assessments for NST-01

	Screening (Day -28 to Day -2)	Check-in (Day -1)	Study Day																		Follow-up Visit (7 to 10 days Post-final Dose)[a]
			Dosing Period 1					Dosing Period 2					Dosing Period 3					Dosing Period 4			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Informed consent	X																				
Inclusion/exclusion criteria	X	X																			
Demographic data (including smoking history)[b]	X																				
Medical history	X	X[c]																			
Height and body weight	X																				
Urinary drug screen	X	X																			
Alcohol breath test	X	X																			
Urine cotinine test	X	X																			
Serology	X																				
Serum follicle-stimulating hormone (FSH)[d]	X																				
Pregnancy test[e]	X	X																			X
Previous medications	X	X																			
Study Residency																					
Check-in		X																			
Check-out																				X	
Non-residential visit	X																				X
Randomisation			X																		
Study drug administration[f]			X					X					X					X			
Pharmacokinetics																					
Blood sampling (plasma)[g]			X	X				X	X				X	X				X	X		

	Screening (Day -28 to Day -2)	Check-in (Day -1)	Study Day																		Follow-up Visit (7 to 10 days Post-final Dose)[a]
			Dosing Period 1					Dosing Period 2					Dosing Period 3					Dosing Period 4			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Cardiodynamic (Continuous) 12-lead Electrocardiogram (ECG)																					
12-lead ECG recordings[h]			X	X				X	X				X	X				X	X		
Safety Assessments																					
Adverse event recording	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead safety ECG[i]	X	X	X					X					X					X			X
Vital signs (blood pressure, pulse rate, and oral body temperature)[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical laboratory evaluations	X	X					X					X					X				X
Full physical examination	X																			X	X
Abbreviated physical examination		X	X					X					X					X			

a. If a subject is withdrawn, 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); efforts will be made to perform all follow-up assessments, if possible. 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 Clinical Research Unit (CRU). The Investigator (or designee) may also request that the subject return for an additional Follow-up Visit.
b. Demographics will also include alcohol and caffeine consumption.
c. Interim medical history.
d. Serum FSH will be performed in all female subjects at Screening to confirm menopausal status.
e. In 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.
f. Doses will be administered on the morning of Days 1, 6, 11, and 16 following an overnight fast (not including water) of at least 8 hours.
g. Blood samples for plasma pharmacokinetic (PK) analysis will be taken at predose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, and 24 hours postdose in each dosing period.
h. Continuous 12-lead ECG recordings will be performed from 1 hour predose to approximately 25 hours postdose in each dosing period. On Days 1, 6, 11, and 16, 12-lead ECGs will be extracted in replicates at 3 timepoints prior to dosing (-45, -30, and -15 minutes) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours postdose. At each extraction timepoint, subjects will be supine for at least 10 minutes prior to and 5 minutes after each timepoint.
i. The 12-lead safety ECGs should be performed after the subject has been in a supine position and at rest for at least 5 minutes. Safety12-lead ECGs will be collected at Screening, Check-in (Day -1), and the Follow-up Visit, and on Days 1, 6, 11, and 16 at predose and 1.5 and 6 hours postdose. At predose on Day 1, 12-lead ECGs will be measured in triplicate at approximately 2-minute intervals. The median value will be used as the baseline value in the data analysis. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges.
j. On Days 1, 6, 11, and 16, vital sign assessments will be taken at predose and 2 hours postdose. Subjects must be supine for no less than 5 minutes before blood pressure and pulse rate measurements.