ITALFARMACO S.p.A.

PROTOCOL 200148

Italfarmaco S.p.A. study number: ITF/2357/54

IND number: CCI

EudraCT number: 2020-003105-63

A RANDOMIZED, PARTIALLY DOUBLE-BLIND, FOUR-PERIOD, FOUR-TREATMENT, CROSSOVER STUDY INVESTIGATING THE PLACEBO-CORRECTED EFFECTS OF A THERAPEUTIC DOSE (100 mg) AND A SUPRATHERAPEUTIC DOSE (300 mg) OF ITF2357 (GIVINOSTAT) AND MOXIFLOXACIN ON QT/QTC INTERVAL IN HEALTHY MALE AND FEMALE SUBJECTS

Contract Research Organization:
Syneos Health Clinique inc.
(« Syneos Health »)

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Protocol Historical File

Version number	Brief description/summary of changes	Date
Final	Signed version.	20-JUL-2020
Amendment I	Version submitted to the IEC.	29-SEP-2020

Changes included in Amendment I:

Changes have been made to amend the duration of some of the on-study restrictions. These changes have been made applied to:

- Page 16, study restrictions under section 2. *Synopsis of Protocol*. The first 5 bulleted points have been changed as follows:
  - "Prescription medication from 14 days prior to the first dosing until after the last PK blood sample collection of the study" now reads "Prescription medication from 14 days prior to the first dosing until study exit"
  - "OTC products from 7 days prior to the first dosing until after the last PK blood sample collection of the study" now read "OTC products from 7 days prior to the first dosing until study exit"
  - "Natural health products from 7 days pre dose until after the last PK blood sample collection of the study" now reads "Natural health products from 7 days prior to the first dosing until study exit"
  - " A depot injection or an implant of any drug within 3 months prior to the first dosing" now reads " A depot injection or an implant of any drug within 3 months prior to the first dosing until study exit"
  - " St. John´s wort from 30 days prior to the first dosing until after the last PK blood sample collection of the study" now reads " St. John´s wort and any drugs known to induce or inhibit hepatic drug metabolism from 30 days prior to the first dosing until study exit"
- Pages 41 to 42, study restrictions under section 9.8 *Study Restrictions*. The third bullet point on page 41, *section 9.8.1 Food and Fluids,* now reads "(…) from 7 days prior to first dosing until study exit", and the first sentence on page 42, *section 9.8.2 Tobacco, Alcohol and Illicit Drugs*, now reads "(…) until study exit."

Clarifications have been made on previous and concomitant medications restrictions; these changes have been made to:

- Page 14, exclusion criteria 18 under section 2. *Synopsis of Protocol.* Point b) of criterion 18) now reads "(…) with the exception of the occasional use of acetaminophen [up to 2 g daily] (…)." Therefore, criteria 19 which explained the occasional use of acetaminophen has been deleted, and criteria 20 which describe the restrictions of plasma donation becomes now criteria 19.

- Page 35, exclusion criteria 18 under section 8.3 *Exclusion Criteria.* Point b) of criterion 18) now reads "(…) with the exception of the occasional use of acetaminophen [up to 2 g daily] (…)." Therefore, criteria 19 which explained the occasional use of acetaminophen has been deleted, and criteria 20 which describe the restrictions of plasma donation becomes now criteria 19.
- Page 16, study restrictions under section 2. *Synopsis of Protocol*:
  - Bullet point 5 has been changed from "St. John´s wort from 30 days prior to the first dosing until after the last PK blood sample collection of the study" to " St. John´s wort and any drugs known to induce or inhibit hepatic drug metabolism from 30 days prior to the first dosing until study exit."
  - Bullet point 10 has been added "Soft or hard drugs or any tobacco or nicotine products from screening until study exit."
- Pages 41 to 42, study restrictions under section 9.8 *Study Restrictions, section 9.8.1 Food and Fluids,* bullet point 4 has been added on page 42 and reads "St John's wort and any drugs known to induce or inhibit hepatic drug metabolism from 30 days prior to first dosing until study exit."
- Page 42, section 9.8.3 concomitant medications, the first sentence now reads "(…) and depot injection or implant of any drug, for the period of time specified in exclusion criterion 18) until study exit."

The urinary pharmacokinetic parameters were updated and now include: $A_{e0\text{-}t}$, R_{max}, T_{Rmax}, and Clr. Therefore changes are brought to:

- Page 19, pharmacokinetics under Section 2, *Synopsis of Protocol*
- Pages 56-57 urine PK parameters under Section 12.2

As a result of the above changes, additional abbreviations and definition of terms have been added or updated; changes have been brought to:

- Pages 20-22 under Section 3 *List of Abbreviations and Definition of Terms*
- Page 56 under Section 12.1 *Plasma PK Parameters*

Signature Page

Sponsor

Italfarmaco S.p.A.

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20092 Cinisello Balsamo
Milan, Italy
Tel.: PPD

Sponsor's representative:

PPD

PPD, MD, PhD

PPD

PPD

Date (XX-MMM-2020)

Signature Page

Contract Research Organization (CRO)

Syneos Health Clinique inc.

PPD

Electronically signed by: PPD
Reason: I am accepting the terms of service
Date: Sep 29, 2020 16:42 EDT

29-Sep-2020

Signature

Date (XX-MMM-2020)

PPD

(printed name and title)

Signature page

Qualified Investigator

I (as Qualified Investigator) have carefully read and understood all sections of this study protocol Final Version dated 20-JUL-2020 and agree that it contains all necessary information required to conduct this study.

I agree to supervise all aspects of the protocol and to conduct the study according to this protocol (including any amendments) and in accordance with the clinical site's Standard Operating Procedures (SOPs), ICH Good Clinical Practice (GCP), all other applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IEC requirements relative to clinical studies and the recommendations laid down in the most recent version of the Declaration of Helsinki.

I will not make changes to the protocol before consulting with Italfarmaco S.p.A. or implement protocol changes without Independent Ethics Committee (IEC) approval except to eliminate an immediate risk to subjects. I agree to administer study drug only to subjects under my personal supervision or the supervision of a sub-investigator. I will not supply the investigational drug to any person not authorized to receive it. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

It is further agreed that the details of this trial and all information provided to me by Italfarmaco S.p.A. or Syneos Health will be held in the strictest confidence and will not be revealed for any reason without written authorization from Italfarmaco S.p.A. except to those who are to participate in the conduct of the trial.

I agree that all data, the case report forms and all materials provided for the conduct of the trial are the property of Italfarmaco S.p.A. unless specified otherwise in writing. It is understood that Italfarmaco S.p.A. will utilize the information derived from this trial in various ways, such as for submission to government regulatory agencies, required internal reports and presentations without violating patient/subject confidentiality in any way.

I further agree that Italfarmaco S.p.A. or its representatives will be permitted access in accordance with current Good Clinical Practice Guidelines, to all data generated by this trial and the source documents from which case report form data were generated.

PPD

Electronically signed by: PPD
Reason: I am accepting the terms of service
Date: Sep 29, 2020 23:31 EDT

Signature

Sep 29, 2020

Date (XX-MMM-2020)

PPD

(printed name and title)

1. Facilities and Responsible Staff

1.1 Clinical Research Facilities

This study will be conducted by Syneos Health at the following facility:

2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Screening and/or return visits (if any) may also be performed at the Montréal Syneos Health facility:

5160, boul. Décarie, suite 800
Montréal (Québec), Canada, H3X 2H9
Tel.: 1-514-485-7500

1.2 Biomedical Laboratory Facilities

Biomedical laboratory testing will be performed by the following laboratories:

Biron Medical Laboratory inc.
4105-F, boul. Matte
Brossard (Québec), Canada, J4Y 2P4
Tel.: 1-514-866-6146

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Syneos Health
5160, boul. Décarie, suite 800
Montréal (Québec), Canada, H3X 2H9
Tel.: 1-514-485-7500

If another biomedical laboratory is used, this will be documented and annexed to the protocol.

1.3 ECG Central Core Laboratory

ERT
150 Allens Creek Rd
Rochester, NY 14618
USA
Tel.: 1- 585-295-7610

PPD , MD PhD
PPD
Tel.: PPD

1.4 Clinical Pharmacology and Regulatory Affairs

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

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1.5 Bioanalytical Facility

Syneos Health
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Table of Contents

2. Synopsis of Protocol

Project No.:	Syneos Health project number: 200148 Italfarmaco S.p.A. study number: ITF/2357/54 IND number: CCI EudraCT number: 2020-003105-63
Study Title:	A RANDOMIZED, PARTIALLY DOUBLE-BLIND, FOUR-PERIOD, FOUR-TREATMENT, CROSSOVER STUDY INVESTIGATING THE PLACEBO-CORRECTED EFFECTS OF A THERAPEUTIC DOSE (100 mg) AND A SUPRATHERAPEUTIC DOSE (300mg) OF ITF2357 (GIVINOSTAT) AND MOXIFLOXACIN ON QT/QTC INTERVAL IN HEALTHY MALE AND FEMALE SUBJECTS
Study Drug:	Givinostat (ITF2357)
Study Phase and Type:	Phase 1 - Thorough QT/QTc Study
Study Objectives:	Primary objective: • To evaluate the effect of a therapeutic dose and a supratherapeutic dose of ITF2357 on the QT/QTc interval. Secondary objectives: • To evaluate the effect of a single oral therapeutic (T) and a supratherapeutic dose (ST) of ITF2357 on other ECG parameters (heart rate [HR], [PR], QRS intervals and T-wave morphology). • To evaluate the pharmacokinetic (PK) parameters of two doses (100 mg and 300 mg) of ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide. • To evaluate the safety and tolerability of two doses (100 mg and 300 mg) of ITF2357.
Study Design:	This will be a single centre, randomized, partially double-blind, single dose, placebo-corrected, 12-sequence, 4-period, crossover study under fasting conditions.
Subjects:	Up to 34 subjects to ensure 28 evaluable healthy adult, males or females, ≥ 18 and ≤ 55 years of age, non-smoker.
Randomization and Blinding:	Treatments T (therapeutic), ST (supratherapeutic), and P (placebo) will be prepared individually by the unblinded team at the site pharmacy, according to the randomization schedule, and will leave the pharmacy room blinded, so that the blinded site team could administer them to the subjects. On the contrary no blinding will be needed with treatment M (moxifloxacin). To maintain blinding for subjects who receive M in Periods 1, 2 and 3, subjects will be randomized to one of 12 treatment sequences. The ECG Central Core Laboratory ECG Analyst, will be blinded to treatment, study period, time point of ECG recording, and subject details such as laboratory results and adverse events (AEs).
Inclusion Criteria:	Subjects must meet all of the following criteria to be included in the study:

	1) Male or female, non-smoker (no use of tobacco or nicotine products within 3 months prior to screening), ≥18 and ≤55 years of age, with BMI >18.5 and <30.0 kg/m^2 and body weight ≥55 kg and ≤100 kg for females and body weight ≥60 kg and ≤100 kg for males. 2) Healthy as defined by: a) The absence of clinically significant illness and major surgery within 4 weeks prior to dosing. Subjects vomiting within 24 hours pre-dose will be carefully evaluated for upcoming illness/disease. Inclusion pre-dosing of the patient in the study is at the discretion of the Investigator, depending on his/her clinical judgement. b) The absence of clinically significant history of neurological, endocrinal, cardiovascular, pulmonary, hematological, immunologic, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease. 3) Non-childbearing potential female defined as: a) Post-menopausal female (absence of menses for 12 months prior to the first study drug administration, bilateral oophorectomy or hysterectomy with bilateral oophorectomy at least 6 months prior to the first study drug administration); or b) Surgically sterile female (hysterectomy or tubal ligation at least 6 months prior to drug administration). 4) Females of childbearing potential who are sexually active with a male partner must be willing to use one of the following acceptable contraceptive methods throughout the study and for at least 90 days after the last study drug administration: a) Simultaneous use of intra-uterine contraceptive device, without hormone release system placed at least 4 weeks prior to study drug administration, and condom for the male partner; b) Simultaneous use of diaphragm or cervical cap with intravaginally applied spermicide and male condom for the male partner, started at least 21 days prior to study drug administration. 5) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a female partner of childbearing potential (childbearing potential females are defined as women that are neither post-menopausal nor surgically sterile) must be willing to use one of the following acceptable contraceptive methods from the first study drug administration until at least 90 days after the last study drug administration: a) Simultaneous use of a male condom and, for the female partner, hormonal contraceptives used since at least 4 weeks or intra-uterine contraceptive device placed since at least 4 weeks; b) Simultaneous use of a male condom and, for the female partner, a diaphragm or cervical cap with intravaginally applied spermicide. 6) Male subjects (including men who have had a vasectomy) with a pregnant partner must agree to use a condom from the first study drug administration until at least 90 days after the last study drug administration.

	7) Male subjects must be willing not to donate sperm until 90 days following the last study drug administration. 8) Female subjects must be willing not to donate ovules until 90 days following the last study drug administration. 9) Subject's written informed consent obtained prior to any study-related procedure; 10) Willingness and capability to comply with the requirements of the study and ability to understand the study procedures and the risks involved. 11) Willing to take out dentures and mouth piercings for study procedures.
Exclusion Criteria:	Subjects to whom any of the following applies will be excluded from the study: 1) Any clinically significant abnormality at physical examination, clinically significant abnormal laboratory test results or positive test for HIV, hepatitis B, or hepatitis C found during medical screening. 2) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 60 or over 90 mmHg, or heart rate less than 40 or over 100 bpm) at screening. For eligibility purposes, not the mean value, but the two single measurements will be considered. 3) Any of the following abnormalities on 12-lead ECG at screening: PR (PR interval) >210 msec; QRS (QRS complex) >120 msec; QTcF (Fridericia's corrected QT interval) >450 msec; any abnormality of cardiac rhythm other than sinus arrhythmia; abnormality of T-wave morphology that will impair the ability to measure the QT interval reliably. The averaged value of three ECGs 5 minutes apart from each other will be used; evaluations have to be used for the evaluation of the QTc interval requested by this exclusion criteria. 4) Subjects with history of sustained and non-sustained cardiac arrhythmias (ECG demonstrated), subjects with a family history of sudden cardiac death and subjects with a history of additional risk factors for torsade de pointes (TdP), heart failure, hypokalemia, and long QT syndrome (LQTS). 5) Any of the following abnormal laboratory test values at screening and baseline Day -1 of Period 1. a) Platelet count <125 x 10^9/L b) Absolute neutrophil count <1.2 x 10^9/L 6) Subjects who have cardiovascular condition such as, but not limited to unstable ischemic heart disease, NYHA Class III/IV left ventricular failure, acute ischemic heart disease in the last year prior to study screening, which may impact the safety of the subject or the evaluation of the result of the study according to the Investigator's judgment; cardiovascular conditions should be discarded based on the results obtained on the ECG, medical examination and routine lab test. 7) Positive urine drug screen, alcohol breath test or urine cotinine test at screening or at baseline (Day -1). 8) History of anaphylaxis reaction or clinically significant drug hypersensitivity reaction (e.g., angioedema, Stevens-Johnson syndrome, Acute Generalized

	Exanthematous Pustulosis, Drug-induced hypersensitivity syndrome, Drug-induced neutropenia). 9) History of allergic reactions to ITF2357, histone deacetylase (HDAC) inhibitors, or other related drugs, moxifloxacin, other quinolones, or to any excipient in the formulation. 10) Positive pregnancy test at screening or at baseline (Day -1). 11) Subjects with a sorbitol intolerance or sorbitol malabsorption or have fructose intolerance. 12) Current or recent (within 3 months of study drug administration) clinically significant gastrointestinal disease that can interfere with drug absorption. 13) Gastrointestinal surgery that interferes with physiological absorption and motility (i.e., gastric bypass, duodenectomy) or gastric bands. 14) History of significant alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to the screening visit (more than 14 units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]). 15) History of significant drug abuse within 1 year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening. 16) Use of ITF2357 for a medical condition or in the context of another clinical trial within a period of 30 days prior to the first dosing. 17) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to the first dosing, administration of a biological product in the context of a clinical research study within 90 days prior to the first dosing, or concomitant participation in an investigational study involving no drug or device administration. 18) Use of medications for the timeframes specified below, with the exception of medications exempted by the Investigator on a case-by-case basis because they are judged unlikely to affect the pharmacokinetic profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption): a) Prescription medications within 14 days prior to the first dosing; b) Over-the-counter (OTC) products and natural health products (including herbal remedies, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to the first dosing, with the exception of the occasional use of acetaminophen (up to 2 g daily); c) Depot injection or implant of any drug within 3 months prior to the first dosing; d) Any drugs known to induce or inhibit hepatic drug metabolism (including St. John's wort) within 30 days prior to the first dosing.

	19) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing. 20) Breast-feeding subject. 21) Inability to be venipunctured and/or tolerate catheter venous access; 22) Inability or difficulty to swallow tablets or suspension. 23) Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study. 24) History or presence of other diseases, metabolic dysfunctions, physical examination findings, or any clinically relevant abnormal laboratory value at screening suggesting an unknown disease and requiring further clinical investigation or which may impact the safety of the subject or the evaluation of the result of the study according to the Investigator's judgment.
Screening Procedures:	Demographic data, medical and medication histories, complete physical examination, body measurements, supine vital signs (blood pressure [BP], heart rate [HR], respiratory rate [RR], and oral temperature [OT]), 12-lead ECG, fasting safety laboratory assessments: including hematology, biochemistry, coagulation (prothrombin time [PT] and partial thromboplastin [PTT]) and urinalysis, serology (human immunodeficiency virus [HIV], hepatitis B and C tests), urine pregnancy test, urine cotinine test, alcohol breath test, urine drug screen, adverse events (AEs) monitoring, and concomitant medications.
Confinements and Washout:	For each period, subjects will be confined for at least 12 hours before dosing until after the 72 hour post-dose assessments. There will be a washout period of 7 days or more between doses.
Study Duration:	The study duration including the screening period will be approximately 8 weeks.
Study Treatments Administrations:	Single oral dose in each period as follows: • Therapeutic dose (T) of ITF2357 100 mg: administered as 1 x 10 mL of ITF2357 10 mg/mL oral suspension + 2 x 10 mL of matching placebo oral suspension (same appearance and taste of ITF2357) under fasting conditions (30 mL in total, for a total ITF2357 dose of 100 mg). • Supratherapeutic dose (ST) of ITF2357 300 mg: administered as 3 x 10 mL of ITF2357 10 mg/mL oral suspension under fasting conditions (30 mL in total, for a total ITF2357 dose of 300 mg). • Placebo (P): administered as 3 x 10 mL of matching placebo oral suspension (same appearance and taste of ITF2357) administered under fasting conditions (30 mL in total). • Moxifloxacin (M): moxifloxacin 400 mg tablet administered as 1 x 400 mg tablet under fasting conditions. No food will be allowed from at least 10 hours before dosing until at least 4 hours after dosing with the exception of one piece of candy for each subject after each dosing due to the bitter taste of the study drug. Except for water given with study medication, no fluids will be allowed from 1 hour before dosing until 1 hour post-dose.

	Subjects will be randomized to receive study treatment in one of the 12 following sequences: **Sequence 1:** T – ST – P – M **Sequence 2:** ST – M – T – P **Sequence 3:** P – T – M – ST **Sequence 4:** M – P – ST – T **Sequence 5:** ST – P – T – M **Sequence 6:** P – M – ST – T **Sequence 7:** T – ST – M – P **Sequence 8:** M – T – P – ST **Sequence 9:** P – T – ST – M **Sequence 10:** T – M – P – ST **Sequence 11:** ST – P – M – T **Sequence 12:** M – ST – T – P
Study Restrictions:	Subjects will be asked to refrain from using products that may potentially affect their safety and/or the PK profile of the study drug. Main study restrictions include: • Prescription medication from 14 days prior to the first dosing until study exit; • OTC products from 7 days prior to the first dosing until study exit; • Natural health products from 7 days prior to the first dosing until study exit; • A depot injection or an implant of any drug within 3 months prior to the first dosing until study exit; • St. John's wort and any drugs known to induce or inhibit hepatic drug metabolism from 30 days prior to the first dosing until study exit; • Food containing poppy seeds within 24 hours prior to admission of each period; • Food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours pre-dose (for subjects who regularly consume large amounts i.e. ≥ 5 cups of coffee/day the restriction will be from one week pre-dose) until after the last PK blood sample collection of each period; • Food or beverages containing grapefruit, starfruit, pomegranate, pineapple, or pomelo from 7 days pre-dose until after the last PK blood sample collection of each period; • Alcohol-based products from 24 hours prior to admission until after the last PK blood sample collection of each period; • Soft or hard drugs or any tobacco or nicotine products from screening until study exit. For safety reasons, subjects will be required to remain in a supine or semi-reclined position and avoid sleeping for the first 4 hours after drug administration.

Blood Sample Collection for Pharmacokinetic Analysis:	ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide, and moxifloxacin will be quantified for PK analyses. A total of 19 blood samples will be collected in each period: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60 and 72 hours post-dose (6 mL for each sampling time for study treatments S, ST, and P; 3 mL for study treatment M). ITF2357 plasma levels will be of interest to correlate with pharmacodynamic (PD) effect. Therefore, only blood samples for study treatments ITF2357 and moxifloxacin will be analyzed. Samples from subjects in the placebo treatment will be saved for analysis if needed. ITF2357 will be analyzed in all subjects, however, ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide will only be analyzed in a subgroup of 12 evaluable subjects (the same population for whom urine samples will be analyzed) as no effect from the metabolites is expected on the QTc results. The samples that would not be analyzed will be kept frozen until bioanalysis results are obtained, if within these results any indication of a possible effect is found, all samples would be further studied.
Urine Sample Collection for Pharmacokinetic Analysis:	ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide be quantified for PK analysis in the same subgroup of 12 evaluable subjects that will also have the analysis of metabolites done in blood samples. Urine samples will be collected in all subjects at 5 times or time intervals during treatment with T, ST, and P: spot pre-dose (within 2 hours before dosing), 0-8 hours, 8-24 hours, 24-48 hours, and 48-72 hours post-dose. Before starting bioanalysis, the biostatistician will randomly select 12 evaluable subjects for whom urine and blood samples will be analyzed for ITF2357 metabolites. The rest of the samples will be kept frozen for further analysis in case any indication is found of a possible effect of the metabolites on the QTc analysis.
Continuous ECG Evaluation:	The cardiodynamic assessment will be performed through 12-lead ECGs extracted from continuous recordings at pre-specified time points, paired with PK samples. For each period, continuous 12-lead ECG recordings will be performed from one hour prior to dosing until approximately 36 hours post-dose. The 12-lead Holter and ECG equipment will be supplied and supported by ERT. All ECG data will be collected using Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12-lead digital recorder. The continuous 12-lead digital ECG data will be stored onto SD memory cards. 12-lead ECGs will be extracted from the continuous recordings at pre-determined time points as defined below, and will be measured centrally by ERT. At the ECG core laboratory, up to 10 replicate ECGs will be extracted at each of the following time points, paired with PK determinations, on Days 1 and 2, in each treatment period: 3 time points prior to dosing (-45, -30, and -15 minutes) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, and 36 hours post-dose. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point, whenever possible. When ECG extractions coincide with vital signs measurements and blood draws, procedures will be carried out in that order.

Subject Safety:	In Periods 1, 2 and 3, a brief physical examination will be performed at check-in on Day -1, and approximately 24 and 72 (Day 4) hours post-dose. In Period 4, a brief physical examination will be performed at check-in on Day -1 and approximately 24 hours post-dose. Body weight: at check-in on Day -1 for each period. Supine BP, HR, RR, and OT: pre-dose and approximately 2, 3, 6, 12 and 24 hours post-dose for each period. 12-lead ECG (safety): pre-dose and approximately 2, 3, 6, 12 and 24 hours post-dose for each period. Alcohol breath test, urine cotinine test, urine drug screen and serum pregnancy test: at check-in on Day -1 for each period. Fasting safety laboratory assesments including hematology, biochemistry, coagulation (PT and PTT), and urinalysis: at check-in on Day -1, 24 and 72 (Day 4) hours post-dose for each period. Medical surveillance: Subjects will be monitored throughout the study by the clinical staff for AEs and concomitant medications. In each period, an Investigator or Sub-investigator, will be on site for drug administration and until 4 hours post-dose, and available on call for the remainder of the study.
Study Exit Procedures/End of Trial Visit/Early Termination Visit:	Complete physical examination, fasting safety laboratory assessments including hematology, biochemistry, coagulation (PT and PTT) and urinalysis, supine vital signs [BP, HR, RR and OT], 12-lead ECG, urine pregnancy test, AEs monitoring, and concomitant medications.
Follow-up Telephone Call (Day 12±2 days):	A follow-up telephone call will be made to all subjects to monitor ongoing AEs or to check for possible AEs on Day 12±2 days after dosing in Period 4.
Analytical Method:	The Bioanalytical Division of Syneos Health will analyze ITF2357, ITF2374, ITF2375, ITF2440, ITF2563, and ITF2955 glucuronide, in plasma and urine samples, and moxifloxacin in plasma samples using validated methods.
Study Endpoints:	Primary endpoint: • The primary endpoint for the cardiodynamic ECG assessment is the placebo-corrected change from-baseline QTcF (ΔΔQTcF). Secondary endpoints: • Change-from-baseline QTcF, PR, QRS, and heart rate (HR) (ΔQTcF, ΔPR, ΔQRS, and ΔHR) • Placebo-corrected ΔPR, ΔQRS and ΔHR (ΔΔPR, ΔΔQRS, and ΔΔHR) • Categorical outliers for QTcF, HR, PR, and QRS • Frequency of treatment emergent changes of T-wave morphology and U-wave presence • The PK endpoints will include the plasma and urine PK parameters for ITF2357, ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide, and moxifloxacin (as described in the Pharmacokinetics section).

	• Safety and tolerability to ITF2357 will be evaluated through the assessment of adverse events (i.e., seriousness, severity, relationship to the study medication, outcome, duration, and management), vital signs, ECG, and clinical laboratory parameters.
Pharmacokinetics:	Parameters for plasma PK: AUC_{0-t}, AUC_{0-12}, AUC_{0-inf}, Residual area, C_{max}, T_{max}, $T_{½\ el}$, and K_{el}, CL/F, and Vd/F. Parameters for urine PK: A_{e0-t}, R_{max}, T_{Rmax}, and Clr.
Statistical Analyses:	<u>Cardiodynamic ECG assessment</u>: The primary analysis will be based on concentration-QTc modeling of the relationship between plasma concentrations of ITF2357 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) >10 msec at clinically relevant plasma levels. In addition, the effects of ITF2357 on the placebo-corrected ΔQTcF, ΔHR, ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will be evaluated at each post-dosing time point ('by time point' analysis). An analysis of categorical outliers will be performed for changes in QTcF, HR, PR, QRS, T-wave morphology and U-wave presence. Assay sensitivity will be evaluated by concentration-QTc analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis. Assay sensitivity will be deemed as met if the slope of the concentration-QTc relationship/ΔQTcF is statistically significant at 10% level of significance in a 2-sided test and the predicted QT effect (i.e. the lower bound of the 2-sided 90% confidence interval [CI] of ΔΔQTcF) is above 5 msec at the observed geometric mean C_{max} of 400 mg moxifloxacin. <u>Pharmacokinetic assessment</u>: Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (arithmetic and geometric means, standard deviation [SD], coefficient of variation [CV%], minimum [Min], maximum [Max], and median) of the plasma concentrations versus time will be presented as well for the pharmacokinetic parameters. <u>Safety assessments</u>: Treatment-emergent adverse events (TEAEs) will be tabulated by treatment for all subjects who were dosed (Safety Population). Changes from baseline values in vital signs, ECG, and clinical laboratory parameters will be evaluated and tabulated by study treatment. Safety and tolerability data will be reported using descriptive statistics. Demographic parameters will be summarized descriptively. <u>Adverse events of special interest</u>: The rates of adverse events of special interest (AESI) should be compared in the treatment and control period. Subgroup analyses should be conducted in terms of age, gender, pre-existing cardiac disease, electrolyte disturbances and concomitant medications. Details of statistical analyses will be developed in a Statistical Analysis Plan (SAP) that will be prepared after completion of the final protocol.

3. List of Abbreviations and Definition of Terms

ADR	adverse drug reaction
AE	adverse event
AESI	adverse events of special interest
Ae_{0-t}	cumulative urinary excretion from time zero to time t
ALT	Alanine Aminotransferase
AMIS	Analytical Methodology Information Sheet
AST	Aspartate Aminotransferase
AUC	area under the curve
b.i.d.	twice a day
BMI	body mass index
BP	blood pressure
CI	confidence interval
CFR	Code of Federal Regulations
Clr	renal clearance
Cl/F	apparent total body clearance
C_{max}	maximum plasma concentration
CRF	Case Report Form
CTA	Clinical Trial Application
CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management System
CV	coefficient of variation
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EDC	electronic data capture
ECG	electrocardiogram
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HDAC	histone deacetylase
HDL	high-density lipoprotein
HEENT	head, eyes, ears, nose, and throat

hERG	human ether-go-go-related gene
HIV	human immunodeficiency virus
HR	heart rate
ICF	Informed Consent Form
IB	Investigator's Brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IC50	concentration at which 50% inhibition observed
ID	identification
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
K_{el}	elimination rate constant
kg	kilogram
L	liter
LDL	low-density lipoprotein
LIMS	Laboratory Information Management System
LQTS	long QT syndrome
M	moxifloxacin
mARC	mitochondrial amidoxime reducing components
Max.	maximum
MedDRA	Medical Dictionary for Regulatory Activities
MDMA	3,4-methylenedioxymethamphetamine
mg	milligram
Min.	minimum
min	minute
mL	milliliter
mmHg	millimeters of Mercury
NOL	No Objection Letter
NYHA	New York Heart Association
OT	oral temperature
OTC	over-the-counter
P	placebo
PCP	phencyclidine
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)

PR	PR interval of the ECG
PTT	partial thromboplastin time
PT	prothrombin time
QA	quality assurance
QC	quality control
QRS	QRS interval of the ECG
QT	QT interval of the ECG
QTcF	Fridericia's corrected QT interval
R_{max}	maximum rate of urinary excretion
RR	RR interval of the ECG
RR	respiratory rate
SAE	serious adverse event
SAP	Statistical Analysis Plan
SARS	severe acute respiratory syndrome
SD	standard deviation
SE	standard error
SOP	Standard Operation Procedure
ST	supratherapeutic dose
SUSAR	suspected unexpected serious adverse reaction
T	therapeutic dose
TEAE	treatment-emergent adverse event
TdP	torsade de pointes
t.i.d	three times a day
$T_{1/2\ el}$	elimination half-Life
T_{max}	time of maximum concentration
T_{Rmax}	time of maximal urinary excretion
US	United States
Vd/F	apparent volume of distribution
WBC	white blood cell
Δ	change from baseline
ΔΔ	placebo-corrected change from baseline

4. Schedule of Events

PROCEDURE	Screening D -28	Periods 1, 2, 3 and 4					Study Exit/Early Termination[1]	Follow-up Telephone Call (Day 12±2 days)
		D-1	D1	D2	D3	D4		
Inform Consent Form	X							
Inclusion and exclusion criteria check[2]		X						
Randomization[2]		X						
Demographic Data[3]	X							
Medical and Medication Histories	X							X
Review and Monitoring of AEs, and Concomitant Medications	X	X	X	X	X	X	X	X
Physical Examination[4]	X	X		X		X	X	
Body Measurements (Height, Weight, and BMI)	X	X[5]						
Supine Vital Signs (BP, HR, RR, OT)	X		X[6]	X[6]			X	
12-lead Safety ECG	X		X[7]	X[7]			X	
Continuous ECG (Holter Monitoring)			X[8]	X[8]				
Hematology	X	X[9]		X[9]		X[9]	X	
Biochemistry	X	X[9]		X[9]		X[9]	X	
Coagulation	X	X[9]		X[9]		X[9]	X	
Serology (HIV and Hepatitis B and C)	X							
Urinalysis	X	X[9]		X[9]		X[9]	X	
Urine Drug Screen	X	X						
Urine Cotinine Test	X	X						
Alcohol Breath Test	X	X						
Serum Pregnancy Test		X						
Urine Pregnancy Test	X					X	X	
Confinement[10]		X	X	X	X	X		
Study Treatment Administration (ITF2357 [T or ST], Moxifloxacin [M] or Placebo [P])[11]			X					
Plasma PK Samples[12]			X	X	X	X		
Urine PK Samples[13]			X	X	X	X		

1 Study procedures performed on Day 4 of Period 4 will serve as study exit procedures. Early termination procedures will be performed as soon as possible after subject withdrawal, always within 14 days after last participation of the subject in the study. If clinically significant abnormal results are found on the laboratory assessments performed during Study Exit/Early Termination, the subject will be invited to the site clinic again to repeat abnormal parameters.

2 Randomization, inclusion and exclusion criteria check to be performed at check-in on Day -1 of Period 1.

3 Demographic data including: age, sex, race, and ethnicity.
4 A complete physical examination (head, eyes, ears, nose, throat (HEENT), neck (including thyroid), chest, lungs, abdomen, back, lymph nodes, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination) will be performed at screening and at study exit. In Periods 1, 2 and 3, a brief physical examination will be performed at check-in on Day -1, and approximately 24 (Day 2) and 72 (Day 4) hours post-dose. In Period 4, a brief physical examination will be performed at check-in on Day -1 and approximately 24 hours post-dose. A brief physical examination will consist of: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject.
5 Body weight, will be determined at check-in on Day -1 of each period.
6 Supine vital signs (BP, HR, RR and OT): pre-dose (Day 1), and approximately 2, 3, 6, 12, and 24 hours post-dose. Vital signs will be matched to the 12-lead safety ECG. Blood pressure measurement: two measures performed after at least 5 minutes of resting in supine position, measures 2 minutes apart from each other.
7 12-lead safety ECG: pre-dose (Day 1) and approximately 2, 3, 6, 12, and 24 hours post-dose. 12-lead safety ECG will be matched to vital signs.
8 Continuous ECG: Subjects will be continuously monitored from approximately 1 hour pre-dose (Day 1) until approximately 36 hours post-dose. Extraction time points for ECG are: -45, -30 and -15 minutes pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, and 36 hours post-dose. The ECG extraction time points will be paired with PK determinations. Subjects will be supinely resting for at least 10 minutes before and 5 minutes after each time point, whenever possible.
9 Fasting safety laboratory assessments (i.e., biochemistry, hematology, urinalysis and coagulation): at check-in on Day -1, and 24 (Day 2) and 72 (Day 4) hours post-dose.
10 Subjects will be confined from at least 12 hours pre-dose on Day -1 until after 72 hour post-dose assessments on Day 4.
11 Study drug administration should be documented along with confirmation of the total dose being administered.
12 Blood samples for ITF2357 and metabolites, and moxifloxacin PK: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60 and 72 hours post-dose (6 mL for each sampling time for study treatments S, ST, and P; 3 mL for study treatment M).
13 Urine samples for PK: spot pre-dose (within 2 hours before dosing), 0-8 hours, 8-24 hours, 24-48 hours, and 48-72 hours post-dose.

5. Introduction

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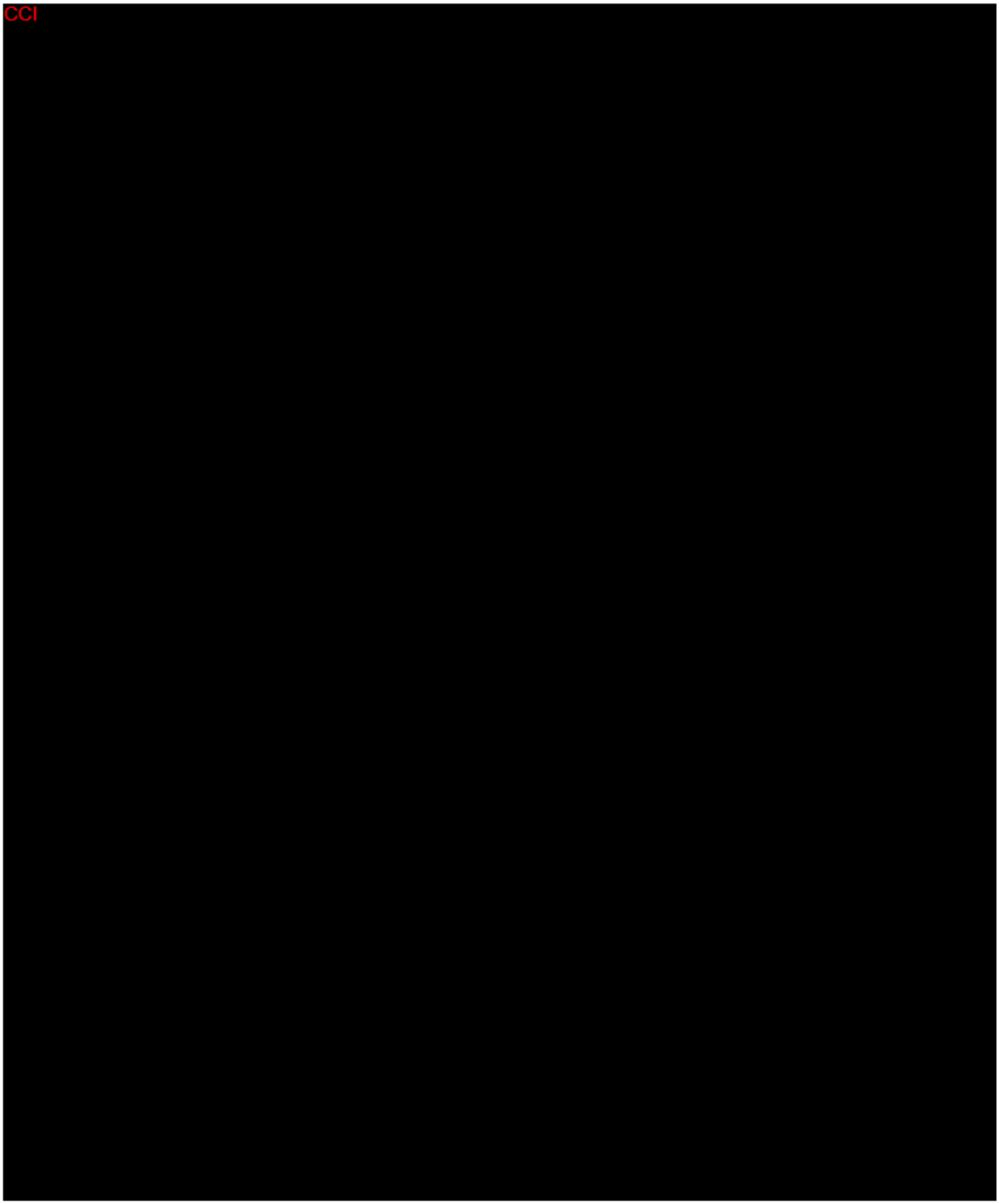

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5.2 Moxifloxacin Background Information

Moxifloxacin is a synthetic fluoroquinolone with a broad spectrum of activity and a bactericidal mode of action. Moxifloxacin is indicated for the treatment of adults with bacterial infections caused by susceptible strains of the designated microorganisms for which treatment is appropriate. The recommended dose for moxifloxacin is 400 mg once daily for all indications. The duration of therapy and route of administration is dependent upon the type and severity of infection.[2]

The bactericidal action results from the interference of moxifloxacin with bacterial topoisomerases II (DNA gyrase) and IV.[2]

Moxifloxacin prolongs the QTc interval, an effect that is believed to occur through inhibition of the I_{Kr} hERG channel in the cardiac myocyte.[3]

Moxifloxacin given as an oral tablet is well absorbed from the gastrointestinal tract. The absolute bioavailability of moxifloxacin is approximately 90%. Following oral administration, peak plasma concentration is attained in 1 to 3 hours. Plasma concentrations increase proportionately with dose up to the highest dose tested (1200 mg single oral dose).[2]

Moxifloxacin is approximately 50% bound to serum proteins, independent of drug concentration. Moxifloxacin is metabolized via glucuronide and sulfate conjugation. The cytochrome P450 system is not involved in moxifloxacin metabolism, and is not affected by moxifloxacin. Moxifloxacin is eliminated from plasma by first-order process. The mean (±SD) elimination half-life from plasma is 12±1.3 hours.[2]

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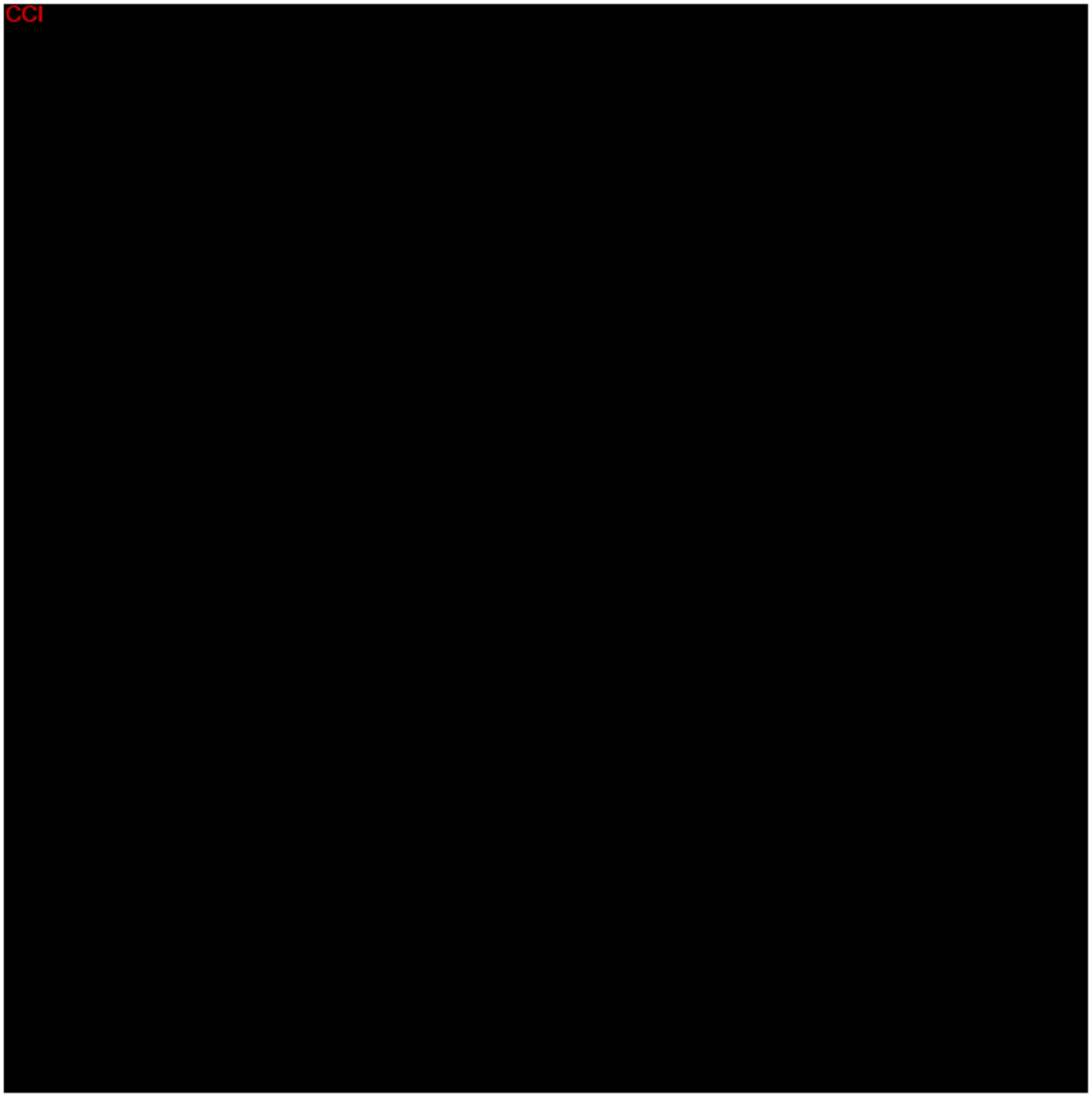

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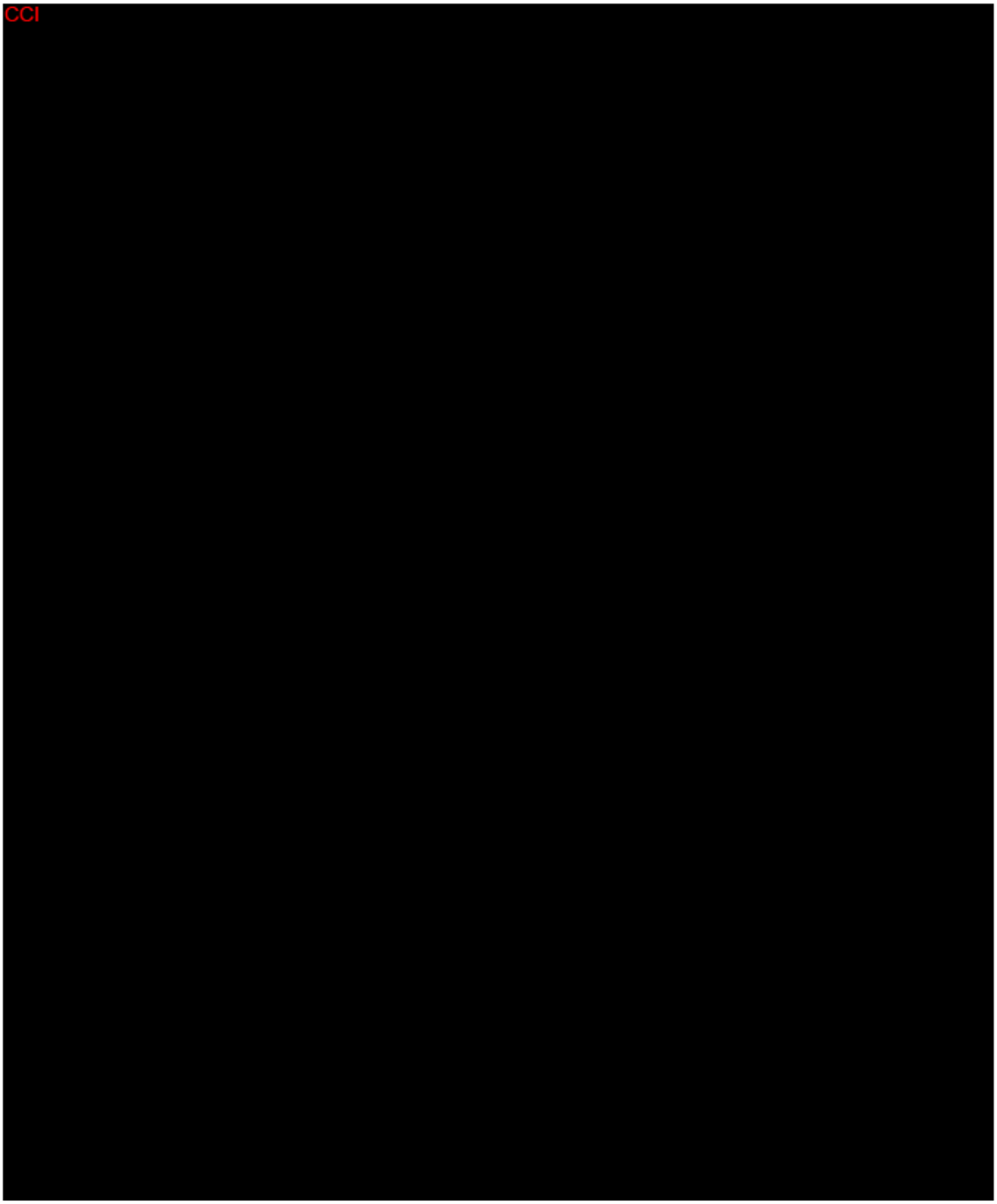

5.3.2 Positive Control Selection

Moxifloxacin 400 mg has been selected as a positive drug control to establish assay sensitivity as moxifloxacin is a reversible blocker of the rapid component of the delayed rectifier, potassium

current of the cardiac K^+ channel. Accordingly, in clinical pharmacology studies, the aggregate mean prolongation of the QTc interval at the expected time of peak plasma concentrations after a single oral dose of 400 mg moxifloxacin was 7±23 msec.[2] Hence, moxifloxacin has been widely used as a positive control agent in studies evaluating effects of investigational products on QT.[2,7,8,9]

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5.3.5 Rationale for the Study Population

In the reproductive toxicity studies, no adverse effects were observed in fertility studies in rats and in embryo-fetal toxicity studies both in rats and in rabbits. However, there are no adequate and well-controlled studies with ITF2357 in pregnant women. Therefore, non-pregnant, non-lactating females will be included in the study. In addition, females of childbearing potential will be included if they use appropriate methods of contraception.

6. Objectives

Primary objective:

- To evaluate the effect of a therapeutic dose and a supratherapeutic dose of ITF2357 on the QT/QTc interval.

Secondary objectives:

- To evaluate the effect of a single oral therapeutic and supratherapeutic dose of ITF2357 on other ECG parameters (HR, PR, QRS intervals and T-wave morphology).
- To evaluate the PK parameters of two doses (100 mg and 300 mg) of ITF2357 and its metabolites: ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide.
- To evaluate the safety and tolerability of two doses (100 mg and 300 mg) of ITF2357.

7. Study Design

This will be a single centre, randomized, partially double-blind, single dose, placebo-corrected, 12-sequence, 4-period, crossover study under fasting conditions.

8. Study Population

8.1 Sample Size

It is planned to enrol up to 34 healthy adults to ensure 28 subjects with evaluable data.

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[redacted]

8.1.1 Sample Size Considerations for Assay Sensitivity

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[redacted]

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[redacted]

8.2 Inclusion Criteria

Subjects enrolled in this study will be members of the community at large. The recruitment advertisements may use various media types (e.g., radio, newspaper, the clinical site Web site and volunteer database). Subjects must meet all of the following criteria to be included in the study:

1) Male or female, non-smoker (no use of tobacco or nicotine products within 3 months prior to screening), ≥18 and ≤55 years of age, with BMI >18.5 and <30.0 kg/m^2 and body weight ≥55 kg and ≤100 kg for females and body weight ≥60 kg and ≤100 kg for males

2) Healthy as defined by:

   a) The absence of clinically significant illness and major surgery within 4 weeks prior to dosing. Subjects vomiting within 24 hours pre-dose will be carefully evaluated for upcoming illness/disease. Inclusion pre-dosing of the patient in the study is at the discretion of the Investigator, depending on his/her clinical judgement.

   b) The absence of clinically significant history of neurological, endocrinal, cardiovascular, pulmonary, hematological, immunologic, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease.

3) Non-childbearing potential female defined as:

   a) Post-menopausal female (absence of menses for 12 months prior to the first study drug administration, bilateral oophorectomy or hysterectomy with bilateral oophorectomy at least 6 months prior to the first study drug administration); or

   b) Surgically sterile female (hysterectomy or tubal ligation at least 6 months prior to drug administration).

4) Females of childbearing potential who are sexually active with a male partner must be willing to use one of the following acceptable contraceptive methods throughout the study and for at least 90 days after the last study drug administration:

   a) Simultaneous use of intra-uterine contraceptive device, without hormone release system placed at least 4 weeks prior to study drug administration, and condom for the male partner;

   b) Simultaneous use of diaphragm or cervical cap with intravaginally applied spermicide and male condom for the male partner, started at least 21 days prior to study drug administration;

5) Male subjects who are not vasectomized for at least 6 months, and who are sexually active with a female partner of childbearing potential (childbearing potential females are defined as women that are neither post-menopausal nor surgically sterile) must be willing to use one of the following acceptable contraceptive methods from the first study drug administration until at least 90 days after the last study drug administration:

   a) Simultaneous use of a male condom and, for the female partner, hormonal contraceptives used since at least 4 weeks or intra-uterine contraceptive device placed since at least 4 weeks;

   b) Simultaneous use of a male condom and, for the female partner, a diaphragm or cervical cap with intravaginally applied spermicide.

6) Male subjects (including men who have had a vasectomy) with a pregnant partner must agree to use a condom from the first study drug administration until at least 90 days after the last study drug administration.

7) Male subjects must be willing not to donate sperm until 90 days following the last study drug administration.

8) Female subjects must be willing not to donate ovules until 90 days following the last study drug administration.

9) Subject's written informed consent obtained prior to any study-related procedure.

10) Willingness and capability to comply with the requirements of the study and ability to understand the study procedures and the risks involved.

11) Willing to take out dentures and mouth piercings for study procedures.

8.3 Exclusion Criteria

Subjects to whom any of the following applies will be excluded from the study:

1) Any clinically significant abnormality at physical examination, clinically significant abnormal laboratory test results or positive test for HIV, hepatitis B, or hepatitis C found during medical screening.

2) Clinically significant vital sign abnormalities (systolic blood pressure lower than 90 or over 140 mmHg, diastolic blood pressure lower than 60 or over 90 mmHg, or heart rate less than 40 or over 100 bpm) at screening. For eligibility purposes, not the mean value, but the two single measurements will be considered.

3) Any of the following abnormalities on 12-lead ECG at screening. PR (PR interval) >210 msec; QRS (QRS complex) >120 msec; QTcF >450 msec; any abnormality of cardiac rhythm other than sinus arrhythmia; abnormality of T-wave morphology that will impair the ability to measure the QT interval reliably. The averaged value of three ECGs 5 minutes apart from each other will be used; evaluations have to be used for the evaluation of the QTc interval requested by this exclusion criteria.

4) Subjects with history of sustained and non-sustained cardiac arrhythmias (ECG demonstrated), subjects with a family history of sudden cardiac death and subjects with a history of additional risk factors for TdP, heart failure, hypokalemia, LQTS).

5) Any of the following abnormal laboratory test values at screening or at baseline (Day -1) of Period 1:

   a) Platelet count $<125 \times 10^9$/L

   b) Absolute neutrophil count $<1.2 \times 10^9$/L

6) Subjects who have cardiovascular condition such as, but not limited to unstable ischemic heart disease, NYHA Class III/IV left ventricular failure, acute ischemic heart disease in the last year prior to study screening, which may impact the safety of the subject or the evaluation of the result of the study according to the Investigator's judgment; cardiovascular conditions should be discarded based on the results obtained on the ECG, medical examination and routine lab test.

7) Positive urine drug screen, alcohol breath test or urine cotinine test at screening or at baseline (Day -1).

8) History of anaphylaxis reaction or clinically significant drug hypersensitivity reaction (e.g., angioedema, Stevens-Johnson syndrome, Acute Generalized Exanthematous Pustulosis, Drug-induced hypersensitivity syndrome, Drug-induced neutropenia).

9) History of allergic reactions to ITF2357, histone deacetylases (HDAC) inhibitors, or other related drugs, moxifloxacin, other quinolones, or to any excipient in the formulation.

10) Positive pregnancy test at screening or at baseline (Day -1).

11) Subjects with a sorbitol intolerance or sorbitol malabsorption or have fructose intolerance.

12) Current or recent (within 3 months of study drug administration) clinically significant gastrointestinal disease that can interfere with drug absorption.

13) Gastrointestinal surgery that interferes with physiological absorption and motility (i.e., gastric bypass, duodenectomy) or gastric bands.

14) History of significant alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to the screening visit (more than 14 units of alcohol per week [1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol]).

15) History of significant drug abuse within 1 year prior to screening or use of soft drugs (such as marijuana) within 3 months prior to the screening visit or hard drugs (such as cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 1 year prior to screening.

16) Use of ITF2357 for a medical condition or in the context of another clinical trial within a period of 30 days prior to the first dosing.

17) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to the first dosing, administration of a biological product in the context of a clinical research study within 90 days prior to the first

dosing, or concomitant participation in an investigational study involving no drug or device administration.

18) Use of medications for the timeframes specified below, with the exception of medications exempted by the Investigator on a case-by-case basis because they are judged unlikely to affect the pharmacokinetic profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption):

    a) Prescription medications within 14 days prior to the first dosing;

    b) OTC products (with the exception of the occasional use of acetaminophen [up to 2 g daily]) and natural health products (including herbal remedies, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to the first dosing;

    c) Depot injection or implant of any drug within 3 months prior to the first dosing;

    d) Any drugs known to induce or inhibit hepatic drug metabolism (including St. John's wort) within 30 days prior to the first dosing.

19) Donation of plasma within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to the first dosing.

20) Breast-feeding subject.

21) Inability to be venipunctured and/or tolerate catheter venous access;

22) Inability or difficulty to swallow tablets or suspension.

23) Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.

24) History or presence of other diseases, metabolic dysfunctions, physical examination findings, or any clinically relevant abnormal laboratory value at screening suggesting an unknown disease and requiring further clinical investigation or which may impact the safety of the subject or the evaluation of the result of the study according to the Investigator's judgment.

9. Clinical Procedures

Unless otherwise specified, procedures, data collection and evaluation will be conducted as per the clinical site SOPs.

9.1 Screening Procedures

Subjects must provide written informed consent prior to initiation of any screening procedures. Subjects provide written consent to general screening procedures, which are not study specific, the consent to those procedures is obtained on a general consent document different from the study specific Inform Consent Form (ICF).

The subjects provide written informed consent to participate in the study, and hence the study specific ICF must be signed and dated by the subject and the Investigator, before participation to

study-specific procedures including any study-specific screening procedures. Both ICF forms must be completed prior to starting any procedures.

Study specific screening procedures will be performed within 28 days preceding administration of study medication, and will consist of: demographic data including age, sex, race, and ethnicity, medical and medication histories, complete physical examination, body measurements, supine vital signs (BP, HR, RR, and OT), 12-lead ECG, fasting safety laboratory assesments including hematology, biochemistry, coagulation (PT and PTT) and urinalysis, serology (HIV, hepatitis B and C tests), urine pregnancy test, urine cotinine test, alcohol breath test, urine drug screen, AEs monitoring, and concomitant medications.

During the screening period, for eligibility purposes, abnormal laboratory or vital signs results may be repeated once if abnormal result is observed at the initial reading. Moreover, abnormalities found in the ECG may need to be confirmed by repeated measurements (usually in triplicate).

In the event that the participation of a subject in the study is delayed, and the screening procedures may become outdated, the subject will then be screen-failed, and re-screened again, under a new screening number and signing a new ICF. The screening procedures will then be repeated.

Each patient will be identified in the study by a patient ID number. Once assigned, the ID subject number must not be reused for any other patient. If the patient fails to be enrolled, the reason for screening failure must be recorded and will be documented in the source data.

9.2 Study Procedures

9.2.1 Check-in (Day -1)

Review of inclusion and exclusion criteria (Period 1 only) except for ECG and vital signs that will be measured before dosing on Day 1, confinement, randomization (Period 1 only), review and monitoring of AEs and concomitant medications, brief physical examination, body weight, fasting safety laboratory assessments including hematology, biochemistry, coagulation (PT and PTT) and urinalysis, urine drug screen, urine cotinine screen, alcohol breath test and serum pregnancy test.

9.2.2 Study Days 1 through 4

Review and monitoring of AEs and concomitant medications, physical examination (Day 2 and Day 4), supine vital signs (Day 1 and Day 2), 12-lead safety ECG (Day 1 and Day 2), continuous ECG (Day 1 and Day 2), fasting safety laboratory assessments including hematology, biochemistry, coagulation (PT and PTT) and urinalysis (Day 2 and Day 4), Urine pregnancy test (Day 4), confinement, study treatment administration (Day 1), plasma PK sample collection and urine PK sample collection.

9.3 Confinements and Washout

For each period, subjects will be confined for at least 12 hours before dosing until after the 72 hour post-dose assessments.

There will be a washout of 7 days or more between doses. The washout period may be increased for logistical considerations. Participation of each subject in this study including the screening period should last approximately 8 weeks.

9.4 Randomization and Blinding

Subjects will be administered each treatment according to the 4-period, 12-sequence, block randomization scheme. Subjects will be randomized to receive study treatment in one of the 12 following sequences:

Sequence	Period 1	Period 2	Period 3	Period 4
1	T	ST	P	M
2	ST	M	T	P
3	P	T	M	ST
4	M	P	ST	T
5	ST	P	T	M
6	P	M	ST	T
7	T	ST	M	P
8	M	T	P	ST
9	P	T	ST	M
10	T	M	P	ST
11	ST	P	M	T
12	M	ST	T	P

ITF2357 (therapeutic [T] and supratherapeutic [ST] dose, and placebo [P]) will be administered in a double-blinded fashion.

Treatments T, ST, and P will be prepared individually by the unblinded team at the site pharmacy, according to the randomization schedule, and will leave the pharmacy room blinded, so that the blinded site team could administer them to the subjects.

On the contrary no blinding will be needed with treatment M.

The ECG Central Core Laboratory ECG Analyst, will be blinded to treatment, study period, time point of ECG recording, and subject details such as laboratory results and AEs.

9.4.1 Study Treatments T, ST, P and M

- Therapeutic dose (T) of ITF2357 100 mg: administered as 1 x 10 mL of ITF2357 10 mg/mL oral suspension + 2 x 10 mL of matching placebo oral suspension (same appearance and taste as ITF2357) under fasting conditions (30 mL in total, for a total ITF2357 dose of 100 mg).

- Supratherapeutic dose (ST) of ITF2357 300 mg: administered as 3 x 10 mL of ITF2357 10 mg/mL oral suspension under fasting conditions (30 mL in total, for a total ITF2357 dose of 300 mg).
- Placebo (P): administered as 3 x 10 mL of matching placebo oral suspension (same appearance and taste of ITF2357) administered under fasting conditions (30 mL in total).

Sponsor, subjects, Investigators, the clinical personnel involved in the collection, monitoring, revision, or evaluation of adverse events, or personnel who could have an impact on the outcome of the study will be blinded with respect to the subject's treatment assignment (ITF2357 [T and ST]) and placebo [P]). Blinding will be maintained until the study has been finalized, the database has been verified, and all protocol violations have been determined. All personnel involved directly in the study shall be unaware of the treatment assigned to the subjects until the database has been closed. The ECG Central Core Laboratory's ECG Analyst, who is different from those who will evaluate laboratory assessments and AEs, will be blinded with respect to the subject's treatment assignment (including moxifloxacin arm), study period, time point of ECG recording and subject details.

The unblinded team at site will comprise the designated pharmacy personnel at the clinical site not directly involved with the clinical aspects of the trial who will prepare and dispense the study medication, will be aware of the randomization code. The study drug and placebo will have the same visual appearance in order to avoid compromising the study blinding.

In the event of an emergency, and only when the knowledge of the treatment assignment is deemed essential for the subject´s care, an envelope for each subject containing his/her treatment assignment will be available from clinical personnel involved with the preparation of the study medication. The Qualified Investigator or other designee delegated by the Qualified Investigator will make every effort to contact the Sponsor prior to unblinding a subject's treatment assignment and will record the date, time, and reason for the unblinding in the study source documents. The Qualified Investigator or designee should immediately inform the study monitor that a treatment code has been opened. Once the study treatment has been unblinded for a given subject, that subject will be definitively withdrawn from the study.

The pharmacy personnel will provide to the Bioanalytical Division of Syneos Health a list of subject numbers to be analyzed, so that only samples from treatments T, ST, and M (moxifloxacin) will be analyzed. Because blood samples from placebo will not be analyzed unless needed, and moxifloxacin will be administered in an open-label basis, a full blinding of bioanalytical staff will not be completely ensured.

The randomization code will not be available to the Bioanalytical Division of Syneos Health until the clinical and analytical phases of the study have been completed.

9.4.2 Study Treatment M

Study treatment M (moxifloxacin) is supplied as a tablet formulation while study treatments T, ST (therapeutic and supratherapeutic doses of ITF2357) and P (matching placebo) are supplied as oral suspension formulations. The positive control, study treatment M (moxifloxacin) will be

administered on an open label basis. The use of a double-blinded positive control is not mandatory,[6] provided that the reading of ECGs is performed in a blinded manner as described above.

9.5 Study Medications

Treatment	Study Medication
Treatment T: Therapeutic dose of ITF2357 100 mg	• ITF2357 10 mg/mL oral suspension – 140 mL/bottle • Placebo oral suspension – 140 mL/bottle Administered as 1 x 10 mL of ITF2357 10 mg/mL oral suspension + 2 x 10 mL of matching placebo oral suspension (same appearance and taste of ITF2357 one) under fasting conditions (30 mL in total, for a total ITF2357 dose of 100 mg).
Treatment ST: Supratherapeutic dose of ITF2357 300 mg	• ITF2357 10 mg/mL oral suspension – 140 mL/bottle Administered as 3 x 10 mL of ITF2357 10 mg/mL oral suspension under fasting conditions (30 mL in total, for a total ITF2357 dose of 300 mg).
Treatment P: Placebo	• Placebo oral suspension – 140 mL/bottle Administered as 3 x 10 mL of matching placebo oral suspension (same appearance and taste of ITF2357 one) administered under fasting conditions (30 mL in total).
Treatment M: Moxifloxacin (Positive Control)	• Moxifloxacin hydrochloride 400 mg tablet Administered as 1 x 400 mg tablet under fasting conditions.

9.6 Drug Supplies and Accountability

It is the responsibility of the Sponsor to ensure that study medication provided for this study are manufactured under Good Manufacturing Practice (GMP) and are suitable for human use. The Sponsor is responsible to ship a sufficient amount of dosage units to allow the clinical site to maintain an appropriate sampling for the study. Study medication will be stored at the clinical site as per applicable requirements.

Moxifloxacin will be purchased directly by Syneos Health and relabeled for clinical use at the site pharmacy.

The medications will be stored in a locked, environmentally-controlled medication room with restricted access. Container(s) will bear a label containing at least the name of the study drug, lot and/or batch number, and manufacturing and/or expiry/retest date.

Individual doses for each subject and period will be dispensed at the clinical site, as per appropriate SOP. Individual doses will be dispensed according to the randomization scheme and appropriate syringes will be labeled with at least the study number, the period number and the subject number/spare number.

All study drug received at the site will be inventoried and accounted for throughout the study and the result recorded in the drug accountability/retention record according to the clinical site appropriate SOP.

Treatment compliance for each period and subject should be recorded in the Clinical Trial Management System (CTMS) and reported in the CRF.

9.7 Study Treatments Administration

Subjects will be required not to wear dentures or oral piercings at the time of dosing.

The ITF2357 and Placebo oral suspensions will be administered by means of graduated dosing syringes for oral use. Before use, the ITF2357 and Placebo oral suspension must be shaken for at least 30 seconds by rotating the bottle by 180° and the homogeneity should be visually verified.

For treatment T, 1 x 10 mL of the ITF2357 10 mg/mL oral suspension and 2 x 10 mL of the matching placebo oral suspension (30 mL in total, for a total ITF2357 dose of 100 mg) will be withdrawn with suitable 10 mL oral syringes provided with the study medications. Three separate syringes will be prepared, 1 with 10 mL of ITF2357 10 mg/mL oral suspension and 2 with 10 mL of matching placebo oral suspension each. The preparation of the syringes will be done and documented at the site pharmacy. The syringes will be packaged in a plastic bag and given to the blinded personnel at the clinical site for their administration to the patients. For administration purposes, the contents of each of the 3 syringes will be transferred to the same glass.

For treatment ST, 3 x 10 mL of ITF2357 10 mg/mL oral suspension (30 mL in total, for a total ITF2357 dose of 300 mg) will be withdrawn with suitable 10 mL oral syringes provided with the study medications. Three separate syringes will be prepared, 3 with 10 mL of ITF2357 oral suspension each. The preparation of the syringes will be done and documented at the site pharmacy. The syringes will be packaged in a plastic bag and given to the blinded personnel at the clinical site for their administration to the patients. For administration purposes, the contents of each of the 3 syringes will be transferred to the same glass.

For treatment P, 3 x 10 mL of the matching placebo oral suspension (30 mL in total) will be withdrawn with suitable 10 mL oral syringes provided with the study medications. Three separate syringes will be prepared, 3 with 10 mL of matching placebo oral suspension each.The preparation of the syringes will be done and documented at the site pharmacy. The syringes will

be packaged in a plastic bag and given to the blinded personnel at the clinical site for their administration to the patients. For administration purposes, the contents of each of the 3 syringes will be transferred to the same glass.

For treatments T, ST and P, the subjects will drink all the suspension immediately after the contents of the 3 syringes is transferred to the same glass. The glass will then be rinsed twice with 105 mL of water and the rinse drunk (total of 210 mL of water).

For treatments T, ST and P the complete preparation and dosing procedure must be completed:

- within 1 hour by the time of starting of study medications syringes preparation
- within 2 minutes by the time the study medication is transferred to the same glass.

For treatment M, the tablet will be administered with 240 mL of water and a hand and mouth check will be performed to ensure consumption of the medication. Time of dosing will be set equal to the time when tablet is administered.

9.8 Study Restrictions

9.8.1 Food and Fluids

No food will be allowed from at least 10 hours before dosing until at least 4 hours after dosing with the exception of one piece of candy for each subject after each dosing due to the bitter taste of the study drug.

Subjects will be in a fasting state before safety laboratory assessments including hematology, biochemistry, coagulation (PT and PTT) and urinalysis.

Meals will be standardized and similar in composition between periods.

Except for water given with study medication, no fluids will be allowed for 1 hour before dosing until 1 hour post-dose. Water will be provided *ad libitum* at all other times.

In addition, subjects will be required to abstain from:

- Food containing poppy seeds within 24 hours prior to admission of each period;
- Food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours pre-dose (for subjects who regularly consume large amounts i.e. ≥ 5 cups of coffee/day the restriction will be from one week pre-dose) until after the last PK blood sample collection of each period;
- Natural health products (including herbal remedies, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) from 7 days prior to first dosing until study exit;

- St John's wort and any drugs known to induce or inhibit hepatic drug metabolism from 30 days prior to first dosing until study exit;
- Food or beverages containing grapefruit, starfruit, pomegranate, pineapple, or pomelo from 7 days prior to dosing until after the last pharmacokinetic blood sample collection of each period.

9.8.2 Tobacco, Alcohol, and Illicit Drugs

Subjects will be required to abstain from using soft or hard drugs or any tobacco or nicotine products from screening until study exit.

Consumption of alcohol-based products will be prohibited from 24 hours prior to admission until after the last pharmacokinetic blood sample collection of each period.

9.8.3 Concomitant Medications

Subjects will be required to avoid using prescription and OTC medications, and depot injections or implant of any drug for the period of time specified in exclusion criterion 18) until study exit. No concomitant drug therapy will be allowed during the study except one(s) required for the medical management of an adverse event. Any concomitant medication use other than the occasional use of acetaminophen (up to 2 g daily) will be evaluated on a case-by-case basis by the Investigator or a Sub-investigator. All concomitant medication use will be documented from screening through study exit/early termination.

9.8.4 Posture and Physical Activity

Subjects will be required to remain in a supine or semi-reclined position but avoid sleeping for the first 4 hours after study treatment administration. However, failure of subjects to comply with these requirements does not constitute a deviation from the protocol if it is medically necessary, required for procedures, or to go to the bathroom. When appropriate, subjects will be accompanied by a staff member while walking. Vigorous activity will be prohibited at all times during the confinement.

9.8.5 Other Restrictions

During the period for which subjects are wearing a Holter monitor, access to laptop, iPod, cell phone, books or TV will be restricted and controlled. In addition, female participants will be required to either remove their bra if any, or wear a bra with no underwire, such as sports bra. If female participants prefer not to wear a bra, it will need to be removed before the placement of electrodes for the continuous recording of their heart rhythm. In both cases, if participant chooses not to wear a bra or wear a wireless bra, it must be for the duration of the entire recording.

9.9 Sample Collection and Processing

During the study, all samples will be collected and processed according to the vendor-specific SOP. At the end of the study and after study report elaboration, samples obtained for clinical laboratory assessments and for PK analysis will be destroyed.

9.9.1 Blood Samples

In each period a total of 19 blood samples will be drawn from each subject for PK analyses. Blood samples for the measurement of ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide, and moxifoxacin will be collected. Only samples from subjects who receive ITF2357 and moxifloxacin will be analyzed, samples from subjects who receive placebo will be saved for analysis if needed.

Samples will be collected pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60 and 72 hours post-dose (6 mL for each sampling time for study treatments S, ST, and P; 3 mL for study treatment M).

ITF2357 will be quantified in all subjects administered with the study drug while the metabolites will be quantified only in a randomized group of 12 subjects, as no effect from the metabolites is expected on the QTc results. The samples that would not be analyzed will be kept frozen until bioanalysis results are obtained, if within these results any indication of a possible effect is found, all samples would be further studied. Blood draws will be performed after the 15-minute supinely resting period around each time point for ECG extraction. The time tolerance window for blood samples collected during the confinement period will be ±10 minutes for all samples. Sample collections done outside the pre-defined time windows will not be considered as protocol deviations since actual post-dose sampling times will be used for pharmacokinetic and statistical analyses. A dead-volume intravenous catheter will be used for blood collection to avoid multiple skin punctures, when appropriate. Otherwise, blood samples will be collected by direct venipuncture.

The total volume of blood including that collected for eligibility and safety purposes should not exceed 435 mL for the whole study.

Before starting bioanalysis, the biostatistician will randomly select 12 evaluble subjects for plasma metabolite analysis. These will be the same 12 evaluable subjects randomely selected for urinary metabolte analysis.

Plasma samples will be collected and processed as per the Analytical Methodology Information Sheet (AMIS).

9.9.2 Urine Sample Collection

During treatment with T, ST, and P, urine will be collected from each subject at 5 times or time intervals: spot pre-dose (within 2 hours before dosing), 0-8 hours, 8-24 hours, 24-48 hours, and 48-72 hours post-dose.

Urine samples will be collected from all subjects, but only urine samples from 12 randomized subjects will be used for the PK analyses of ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563, and ITF2955 glucuronide. The rest of the samples will be kept frozen for further analysis in case any indication is found of a possible effect of the metabolites on the QTc analysis.

If a subject cannot void his or her bladder within 30 minutes before dosing, a sample from earlier that morning may be used as the pre-dose sample. Voids that occur within the time interval will

be pooled, and subjects will be asked to void their bladder within 30 minutes before the end of each collection interval, so that each new interval will begin with an empty bladder. Any urine voided by subjects at the intersection (within 10 minutes) of two intervals will be included in the earlier sample. Any urine voided by subjects but not collected will be documented.

Before starting bioanalysis, the biostatistician will randomly select 12 evaluble subjects for urinary metabolite analysis. These will be the same evaluable subjects randomely selected for plasma metabolte analysis.

Urine samples will be collected and processed as per the AMIS.

9.10 Subject Monitoring

Subjects will be monitored throughout the study by the clinical staff for AEs. In each period, an Ivestigator or Sub-investigator will be on site for drug administration and until 4 hours post-dose, and available on call for the remainder of the study. If necessary, an Investigator or designee, at the clinical site or a healthcare professional in a nearby hospital will administer treatment for any adverse event(s). A crash cart or emergency bag containing the necessary rescue material and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

Safety parameters, including laboratory results and ECG, will be assessed by an Investigator or Sub-investigator, using the clinical site's criteria for biomedical laboratory and ECG acceptance ranges as suggested guidelines in making the medical assessment.

Scheduled safety measurements will be repeated according to the clinical site SOPs or upon request from an Investigator or Sub-investigator. Any abnormal repeated measurement will be evaluated by an Investigator or Sub-investigator and repeated if judged necessary. Further action may be taken upon an Investigator or Sub-investigator's request.

Subjects will be advised to notify their health care professional(s) (e.g., physician, dentist, and/or pharmacist) that they are participating in a clinical research study on a drug called ITF2357 (Givinostat), which is being developed by Italfarmaco S.p.A. for potential clinical use in patients suffering from Muscular Dystrophies including Duchenne and Becker Muscular Dystrophy, and chronic myeloproliferative neoplasms including Polycythemia Vera, before taking any medicines or undergoing any medical procedure.

9.10.1 Vital Signs

BP, HR, RR, and OT will be measured in a supine position at: screening, pre-dose and aproximately 2, 3, 6, 12 and 24 hours post-dose for each period, and at study exit. Vital signs will be matched to the safety ECG (at screening, pre-dose and approximately 2, 3, 6, 12, and 24 hours post-dose for each period, and at study exit). When vital signs measurements coincide with ECG or blood draw, the assessments will be performed in the following order: ECG, vital signs, and blood draw. Urine collection timepoints will not be prioritized over safety and other PK assessments.

For the measurement of BP, two measures performed after at least 5 minutes of resting in supine position should be taken 2 minutes apart from each other.

9.10.2 Safety ECG

Supine 12-lead ECG will be performed at screening, pre-dose and approximately 2, 3, 6, 12 and 24 hours post-dose for each period, and at study exit. Safety ECG will be matched to vital signs (at screening, pre-dose and 2, 3, 6, 12 and 24 hours post-dose for each period, and at study exit). When ECG coincides with vital signs measurements or blood draw, the assessments will be performed in the following order: ECG, vital signs, and blood draw. Urine collection timepoints will not be priortized over safety and other PK assessments.

9.10.3 Continuous ECG Recording

The cardiodynamic assessment will be performed through 12-lead ECGs extracted from continuous recordings at pre-specified time points, paired with PK samples.

Continuous 12-lead ECG recordings will be performed from one hour prior to dosing in each treatment period to 36 hours post-dose. The 12-lead Holter and ECG equipment will be supplied and supported by ERT. All ECG data will be collected using Global Instrumentation (Manlius, NY, USA) M12R ECG continuous 12-lead digital recorder. The continuous 12-lead digital ECG data will be stored onto SD memory cards. 12-lead ECGs will be extracted from the continuous recordings at pre-determined time points as defined in Section 4. Schedule of Events, and will be measured centrally by ERT.

Subjects will be supinely resting for at least 10 minutes prior to and 5 minutes after each time point for ECG extractions, whenever possible. Subjects will be required to avoid postural changes during these ECG recordings.

When time points for ECG extractions coincide with vital signs measurements, and blood draws, procedures will be carried out in that order. Urine collection timepoints will not be priortized over safety and other PK assessments.

At the ECG core laboratory, up to 10 replicate ECGs will be extracted at each of the following time points on Day 1 in each treatment period: 3 time points prior to dosing (-45, -30 and - 15 minutes) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, and 36 hours post-dose.
The following principals will be followed in ERT's core laboratory:

- 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 analyzed by the same reader
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

The following is a brief description of ECG analysis methods utilized by ERT's core laboratory.

9.10.3.1 TQT Plus ECG Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the 'TQT Plus method', a computer-assisted and statistical process utilized by ERT. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (e.g., 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).

9.10.3.2 Expert-Precision QT Analysis

Expert-precision QT analysis will be performed on all analyzable (non-artifact) 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.

Measurements of all primary ECG parameters (QT, QTc, 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 QC assessment is performed by a cardiologist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is calculated, and then the mean of all available medians from a nominal timepoint is 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 timepoint. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) is electronically marked.

For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, i.e., changes not present at baseline. For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in any replicates at the time point. For baseline, the category will be deemed as present if observed in any replicates from all time points that constitute baseline. The T-wave morphology categories are described as follows.

Table 1: T-wave morphology and U-wave presence categories (assessed manually)

Category	Description
Normal T-wave	Any positive 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

Category	Description
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive/negative and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave
U-waves	Presence of abnormal U-waves

9.10.4 Physical Examination

A complete physical examination will be performed at screening and at study exit. A complete physical examination includes assessments of the following: head, eyes, ears, nose, throat (HEENT), neck (including thyroid), chest, lungs, abdomen, back, lymph nodes, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination.

In Periods 1, 2 and 3, a brief physical examination will be performed at check-in on Day -1, and approximately 24 and 72 (Day 4) hours post-dose. In Period 4, a brief physical examination will be performed at check-in on Day -1 and approximately 24 hours post-dose. A brief physical examination includes assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject.

Body measurements including body height, body weight and BMI will be measured at screening. Body weight will be measured at check-in (Day -1) for each period.

9.10.5 Drug, Alcohol, and Cotinine Screen

A urine drug screen (amphetamines, methamphetamines, barbiturates, benzodiazepines, tetrahydrocannabinol, cocaine, opiates, PCP, MDMA, methadone), an alcohol breath test, and a urine cotinine test, will be performed at screening. A urine drug screen, an alcohol breath test, and a urine cotinine test and will also be performed before dosing of each period.

9.10.6 Pregnancy Test

A urine pregnancy test will be performed at screening and at study exit, and a serum pregnancy test will be performed at check-in of each period.

9.10.7 Laboratory Assessments

9.10.7.1 Hematology

Fasting hematology will be performed at screening, before dosing of each period (at check-in or in the morning of Day -1), 24 (Day 2) and 72 (Day 4) hours post-dose, and at study exit. The following will be assessed: complete blood count with differential, hemoglobin, and hematocrit.

9.10.7.2 Biochemistry

Fasting biochemistry will be performed at screening, before dosing of each period (at check-in or in the morning of Day -1), 24 (Day 2) and 72 (Day 4) hours post-dose, and at study exit. The following will be assessed: cholesterol [total, LDL and HDL], triglycerides, albumin, alkaline phosphatase, AST, ALT, urea, cystatin C, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, and total protein.

9.10.8 Coagulation

Fasting coagulation will be performed at screening, before dosing of each period (at check-in or in the morning of Day -1), 24 (Day 2) and 72 (Day 4) hours post-dose, and at study exit. The following will be assessed: PT and PTT.

9.10.8.1 Serology

Serology will be performed at screening. The following will be assessed: HIV antigen and antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody.

9.10.8.2 Urinalysis

Fasting urinalysis will be performed at screening and before dosing of each period (at check-in or in the morning of Day -1), 24 and 72 hours post-dose, and at study exit. The following will be assessed: macroscopic examination, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood, nitrite, urobilinogen, and leukocytes. Unless otherwise specified, microscopic examination will be performed on abnormal findings.

9.11 Study Exit Procedures (End of Trial Visit/Early Termination Visit)

This visit is performed at the end of the subject participation in the trial, upon completion on Day 4 of Period 4. Study procedures performed on Day 4 of Period 4 will serve as study exit procedures. This visit is also performed in case of early termination or withdrawals, in this case, study procedures will be done the same day of withdrawal or as soon as possible but always within 14 days after last participation of the subject in the study. If clinically significant abnormal results are found on the laboratory assessments performed during Study Exit/Early Termination, the subject will be invited to the site clinic again to repeat abnormal parameters.

The following procedures should be performed: complete physical examination, fasting safety laboratory assessments including hematology, biochemistry, coagulation (PT and PTT), and urinalysis, supine vital signs [BP, HR, RR and OT], 12-lead ECG, urine pregnancy test, AEs monitoring, and concomitant medications.

9.12 Follow-up Telephone Call (Day 12±2 days):

A follow-up telephone call will be made to all subjects to monitor ongoing AEs or to check for possible AEs on Day 12±2 days after dosing in Period 4.

9.13 Data Collection and Evaluation

Subjects' personal information will be stored in an electronic data capture (EDC) system (Initiator™ or Alphadas®). All clinical raw data will be recorded promptly, accurately, and legibly; either directly into the EDC system as e-source data or indelibly on paper (e.g., raw data sheets when electronic data capture is not possible). A detailed list of the type (electronic or paper) and location for all source data will be included in the Trial Master File. When recorded electronically, Case Report Forms will be electronically generated afterwards. All raw data will be conserved in order to maintain data integrity. The Qualified Investigator and designee have the responsibility of ensuring the completeness and accuracy of the clinical data.

All laboratory results provided by Biron biomedical laboratory will be stored in InLab (Clinical Laboratory Information Management System). Initiator™, Alphadas®, and InLab are validated and are Code of Federal Regulations (CFR) part 11 compliant applications.

Clinical data management will be performed in accordance with applicable Syneos standards and data cleaning procedures to ensure the integrity of the data, e.g., removing errors and inconsistencies in the data. Adverse events and concomitant medication terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

9.14 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or a Sub-investigator may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with the clinical site's SOP:

- safety reason;
- non-compliance with protocol requirements;
- significant protocol deviation;
- positive alcohol breath test, cotinine test, drug screen, or pregnancy test;
- vomiting within 4 hours after dosing;
- marked prolongation of the QT/QTc interval (increases in QT/QTc to >500 ms or of >60 ms over baseline);
- unblinding of subjects treatment

Hematology, biochemistry, coagulation (PT and PTT), and urinalysis results will be reviewed by an Investigator or Sub-investigator prior to dosing; subjects will be withdrawn from the study if it is deemed that the subject's safety may be at risk on the basis of these test results.

Subjects excluded from dosing in one period as per criteria listed above, may be invited to participate in subsequent periods of the study if deemed appropriate by the Investigator and appropriate from a statistical standpoint (i.e. would permit adequate statistical comparison). However, subjects with positive alcohol, cotinine, drug screen, or pregnancy test and subjects withdrawn due to unblinding of the study treatment, will be definitively withdrawn from the study.

Subjects who withdraw or are withdrawn from the study after dosing will not be replaced. However, in the event that the number of drop-outs exceeds initial expectations, subjects who withdraw or are withdrawn might be replaced at the discretion of the Sponsor. Such replacement resulting in dosing more subjects than planned in this protocol would be documented in a protocol amendment.

Subjects who withdraw or are withdrawn will be asked to remain at the clinic until the Investigator or Sub-investigator agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped. A PK blood draw may be collected at the time of withdrawal if deemed required by the Investigator. Study exit procedures/early termination visit will be performed at the time of withdrawal from the study or as soon as possible thereafter.

9.15 Adverse Events and Adverse Reactions

Adverse Event:

An AE is "any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment (International Conference on Harmonisation [ICH] E2A)." Study drug includes both the investigational drug under evaluation and the comparator drug or placebo. Medical conditions that were present before starting study drug are only considered AEs if they worsen after the subject has started the study drug.

Adverse Drug Reaction:
In the pre-approval clinical experience with a new medicinal product: "all noxious and unintended responses to a medicinal product related to any dose should be considered an Adverse Drug Reaction (ADR)." The phrase "responses to a medical product" means that a causal relationship between a medical product and an AE is at least a reasonable possibility, i.e., the relations cannot be ruled out.

Unexpected Adverse Drug Reaction:

An unexpected ADR is an ADR, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).

9.15.1 Serious Adverse Event (SAE)

A SAE is any event that meets any of the following criteria:

- Death
- Life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity
- Congenital anomaly/birth defect in the offspring of a subject

- Other: Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are:
  - Intensive treatment in an emergency room or at home for allergic bronchospasm
  - Blood dyscrasias or convulsions that do not result in inpatient hospitalization
  - Development of drug dependency or drug abuse

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is referred to an ADR that complies with both the definitions of "serious" and "unexpected."

Any abnormal laboratory test results felt to be clinically significant in the medical and scientific judgment of the Investigator are to be recorded as AEs or SAEs. Subject's hematological parameters, in particular platelet counts, should be carefully monitored. Subjects should further be carefully monitored for signs of gastrointestinal AE's and, if necessary.

9.15.2 Recording of Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study medication. Adverse events will be collected and documented during the course of the study, from ICF signature until a period of 12±2 days following the last study drug administration.

Adverse events will be followed-up until complete resolution, or until the Investigator judges it to be safe to discontinue follow-up. The relationship to the study medication will be classified by the Investigator according section 9.15.4.

9.15.3 Assessment of Severity

The severity of AEs will be assessed and graded according to the most recently published National Cancer Institute Common Terminology Criteria for AE (CTCAE).

9.15.4 Assessment of Relationship to the Study Drug

The relationship with the study drug should be assessed as:

- related to study drug;
- not related to study drug;
- unknown.

The assessment of the relationship of an adverse event with the administration of study drug is a clinical decision based on all available information at the time of the completion of the CRF.

- An assessment of "Related" indicates that there is a reasonable suspicion that the AE is associated with the use of the study drug.

- An assessment of "Not related" would include the existence of a clear alternative explanation, or non-plausibility.
- An assessment "Unknown" indicates there is not a reasonable suspicion that the AE is associated with the use of the study drug and at the same time there is not the existence of a clear alternative explanation or non-plausibility. In this case, Investigator has to collect all possible information in order to assess the relationship with the study drug, particularly in case of SAEs.

9.15.5 Serious Adverse Events Reporting

Any SAE that occurs after a subject has signed the Informed Consent Form must be reported by the Investigator to the Sponsor within 24 hours of learning of its occurrence.

The Investigator must notify the Sponsor of the SAE by completing the word version of the SAE reporting form and e-mailing the pdf version to the addresses specified below.

Serious adverse event reports must be made whether or not the Investigator considers the event to be related to the investigational drug.

The Investigators are required to complete the SAE Form. Sufficient details must be provided to allow for a complete medical assessment of the SAE and independent determination of possible causality. The Investigators are obliged to pursue and provide additional information as requested by Italfarmaco S.p.A. or its designee. The notification must be directed to:

Italfarmaco S.p.A.

Corporate Drug Safety
Via dei Lavoratori, 54
20092 Cinisello Balsamo (MI), Italy
Phone: PPD
Fax: PPD
Fax (back-up): PPD
Mobile: PPD
e-mail: drug-safety@italfarmaco.com

The same procedure must be applied to the SAE follow-up information.

9.15.6 Suspected, Unexpected, Serious Adverse Drug Reactions

9.15.6.1 Fatal or Life-threatening Serious, Unexpected Adverse Drug Reactions

The Sponsor is responsible for notifying regulatory agencies of fatal or life-threatening serious, unexpected adverse drug reactions (by telephone, facsimile transmission or in writing) as soon as possible, but no later than 7 calendar days after becoming aware of the information. Additionally, within 8 days after having informed the agency(ies), a complete report must be submitted, including an assessment of the importance and implication of any findings. Syneos Health will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

It is the responsibility of the clinical site to report as soon as possible, but no later than 7 calendar days after first knowledge by the Investigator, fatal or life-threatening serious, unexpected adverse drug reactions to the IEC responsible for the study.

9.15.6.2 Other Suspected, Unexpected, Serious Adverse Drug Reactions

The Sponsor is responsible for notifying regulatory agencies of all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening as soon as possible, but no later than 15 calendar days after becoming aware of the information. Syneos Health will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

It is the responsibility of the clinical site to report to the IEC responsible for the study all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening, as soon as possible, but no later than 15 calendar days after first knowledge by the Investigator.

9.15.7 Adverse Events of Special Interest

AESI are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event). AESI for this study include the following:

- Torsade de pointes
- Sudden death
- Ventricular tachycardia
- Ventricular fibrillation and flutter
- Post-dose syncope
- Seizures

Which are to be classified as AESI in the CRF and will be recorded from ICF signature and throughout the study period until study completion (follow-up telephone call). When these events are identified they should be examined closely for other risks factors, and evaluation and the need for evaluation by a cardiac specialist will be discussed by the Investigator and the Sponsor

9.16 Pregnancy

In the event a dosed female subject (or the female partner of a dosed male subject, when applicable) becomes pregnant during the treatment phase or through 3 months after the last dose of study drug, this pregnancy must be reported to the site personnel immediately.

The Investigator is required to report within 24 hours the pregnancy, using the Pregnancy Notification Form, to Italfarmaco Corporate Drug Safety by mailing to the following e-mail address:

Italfarmaco Corporate Drug Safety: drug-safety@italfarmaco.com

If notification cannot be made via these means due to technical delivery problems, initial notification may be made by telephone or by fax, using the following numbers:

Italfarmaco Corporate Drug Safety:
Phone: PPD
Fax: PPD
Fax (back-up): PPD

Follow-up information regarding the course and outcome of the pregnancy with an onset within the above defined time frame, will be documented (after obtaining the consent of the female partner, when applicable) as per site's SOP. Any premature termination should be reported, and the status of the mother and child should be reported to the sponsor after delivery. If the outcome of the pregnancy meets the criteria for classification as an SAE, reporting of the event to the IEC and regulatory agency(ies) will be performed as per site's SOP.

Any subject who becomes pregnant during the study will be immediately withdrawn.

9.17 Overdose

In general, a drug overdose in a clinical study is defined as the accidental or intentional use of a drug or medicine in an amount exceeding the protocol defined dose. The Investigator must immediately notify the Sponsor of any occurrence of overdose with study drug.

Any overdose or incorrect administration of study drug should be recorded in the CTMS and reported on the relevant study drug administration Case Report Form (CRF) and the Protocol Deviation CRF. AEs associated with an overdose or incorrect administration of study drug should be reported on the relevant Adverse Event CRF. No safety data related to overdosing of ITF2357 are available.

Any instance of overdose (suspected or confirmed, with and without an AE) must be reported to the Sponsor within 24 hours and, only in case of AEs, it must be fully documented as a SAE. Details of any signs or symptoms and their management should be recorded in the SAE Form including details of any antidote or systematic treatment administered. Any signs or symptoms of over-dosage will be treated symptomatically.

Any other situations putting the subject at risk of an adverse reaction, such as misuse and abuse, medication errors, suspect of transmission of infective agents must be reported to the Sponsor within 24 hours and be fully documented as a SAE.

MedDRA for coding event and WHO Drug Dictionary for coding concomitant medications will be used within the Italfarmaco safety database.

9.18 Reportable Disease

In the case a subject has or manifests any clinical signs characteristic of a reportable disease or condition (e.g., HIV, tuberculosis, SARS), it is the responsibility of the Qualified Investigator or designee to notify the Public Health authorities within 48 hours after becoming aware of the information.

9.19 Premature Termination of the Study

The study may be prematurely terminated by the Qualified Investigator following consultation with the Sponsor, by the Sponsor or by the regulatory authorities. Following a decision to discontinue the trial, the Qualified Investigator or designee will promptly inform the active study subjects and the IEC responsible for this trial, stating the reasons for discontinuation of the study and, furthermore, advise them in writing of any potential risks to the health of study subjects or other persons. It is the Sponsor's responsibility to report the premature termination of the study to the regulatory authority(ies), when required by the applicable regulatory requirement(s). The Canadian regulatory agency must be informed of premature termination within 15 days, provided with the reasons for the trial discontinuation and of any potential risks to the health of study subjects or other persons. Syneos Health may notify the Canadian regulatory agency on behalf of the Sponsor upon his request.

10. Analytical Methodology

When applicable, samples will be transported to the bioanalytical facility in at least two separate shipments, with each set of aliquots in separate shipments. Once the bioanalytical laboratory confirms receipt of the first shipment, the second set of aliquots may be sent. The samples should be packed on sufficient dry ice to keep them frozen for at least 72 hours.

The Bioanalytical Division of Syneos Health will analyze ITF2357, ITF2374, ITF2375, ITF2440, ITF2563, and ITF2955 glucuronide, in plasma and urine samples, and moxifloxacin in plasma samples using validated methods.

Analyst and Watson LIMS (Laboratory Information Management System) will be used at different steps of the analysis.

The bioanalytical work in support of the study will be conducted in compliance with the GCP, using the SOPs in place in the Bioanalytical Division of Syneos Health. These SOPs are in accordance with applicable regulations in the industry: Guidelines on Bioanalytical Method Validation, Good Laboratory Practice (GLP), and Guideline for GCP ICH E6 (R2).

Samples from subjects included in the Pharmacokinetic Population (see section 12.3.3) and from subjects who were withdrawn from the study due to adverse events, or vomiting episodes, will be analyzed.

11. Study Endpoints

Primary endpoint:

- The primary endpoint for the cardiodynamic ECG assessment is the placebo-corrected change from-baseline QTcF (ΔΔQTcF).

Secondary endpoints:

- Change-from-baseline QTcF, PR, QRS, and heart rate (HR) (ΔQTcF, ΔPR, ΔQRS, and ΔHR)

- Placebo-corrected ΔPR, ΔQRS and ΔHR (ΔΔPR, ΔΔQRS, and ΔΔHR)
- Categorical outliers for QTcF, HR, PR, and QRS
- Frequency of treatment emergent changes of T-wave morphology and U-wave presence
- The PK endpoints will include the plasma and urine PK parameters for ITF2357, ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide, and moxifloxacin (as described in the Pharmacokinetics section).
- Safety and tolerability to ITF2357 will be evaluated through the assessment of adverse events (i.e., seriousness, severity, relationship to the study medication, outcome, duration, and management), vital signs, ECG, and clinical laboratory parameters.

12. Pharmacokinetic and Statistical Analyses

Pharmacokinetic analysis will be performed using Phoenix® WinNonlin®. Inferential statistical analyses will be performed using SAS® according to FDA and EMA guidelines.

12.1 Plasma PK Parameters

The following pharmacokinetic parameters will be calculated by standard non-compartmental methods for ITF2357 and metabolites: ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide, and moxifloxacin in plasma:

1) AUC_{0-t}: area under the concentration-time curve from time zero to the last measurable concentration
2) AUC_{0-12}: area under the concentration-time curve from time zero to 12 hours
3) AUC_{0-inf}: area under the concentration-time curve from time zero to infinity (extrapolated)
4) C_{max}: maximum observed concentration
5) Residual area: calculated as $100*(1- AUC_{0-t} / AUC_{0-inf})$
6) T_{max}: time of observed Cmax
7) $T_{½\ el}$: elimination half-life
8) K_{el}: elimination rate constant
9) CL/F: apparent total body clearance, calculated as $Dose/AUC_{0-inf}$
10) Vd/F: apparent volume of distribution, calculated as $Dose/K_{el} \times AUC_{0-inf}$

12.2 Urine PK Parameters

The following PK parameters will be calculated for ITF2357 and metabolites ITF2374, ITF2375, ITF2440, ITF2563 and ITF2955 glucuronide ITF2955 in urine:

1) Ae_{0-t}: Cumulative urinary excretion from time zero to time t, calculated as the sum of the amounts excreted over each collection interval. The amount excreted in urine for each time interval is calculated as the urine concentration multiplied by the urine volume.

2) R_{max}: Maximum rate of urinary excretion, calculated by dividing the amount of drug excreted in each collection interval by the time over which it was collected

3) T_{Rmax}: Time of R_{max}, calculated as the midpoint of the collection interval during which R_{max} occurred

4) Clr: Renal clearance, calculated as Ae_{0-t} / AUC_{0-t} (plasma)

Additional PK analysis may be performed. Upon Sponsor's request, pharmacokinetic repeats might be performed according to Syneos Health's SOP. If re-assays are requested for pharmacokinetic reasons, final results will include re-assay values, while results with original values will be presented in an appendix of the clinical study report as supportive data.

12.3 Analysis Populations

12.3.1 Safety Population

The Safety Population will include all subjects who receive at least 1 dose of study drug (therapeutic and supratherapeutic doses of ITF2357, moxifloxacin, or placebo).

12.3.2 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 one post-dose time point with a valid ΔQTcF value. The QT/QTc Population will be used for the by-time point and categorical analyses of the cardiodynamic ECG parameters.

12.3.3 Pharmacokinetic Population

The PK population will include all subjects completing at least 3 periods, including at least T, ST, and M and for whom the PK profile can be adequately characterized. The pharmacokinetic population will be used for calculation of PK parameters and statsitical analyses.

Any subject with pre-dose concentrations will be excluded from the PK and statistical analysis for the respective analyte for the concerned period if the pre-dose concentration is greater than 5% of the C_{max} value of that period for that subject.

Data from subjects who experienced emesis during the sampling interval and who were not withdrawn as per criterion established under section 9.14 may be evaluated after completion of the PK analysis.

Any subject who experienced emesis within 2 times median T_{max} of the current study will be excluded from the statistical analysis.

Data (concentrations and PK parameters) from subjects excluded due to a pre-dose concentration greater than 5% of their C_{max} or from subjects withdrawn due to adverse events or vomiting episodes will be presented but excluded from descriptive statistics for the concerned period.

12.3.4 Pharmacokinetic Concentration Population

The PK concentration population will include all subjects who receive a dose of ITF2357 or moxifloxacin and provide at least one evaluable PK concentration for ITF2357 or moxifloxacin.

12.3.5 PK/QTc Population

The PK/QTc Population will include all subjects who are in both the QT/QTc and PK Concentration populations with at least one pair of post-dose PK and QTcF data from the same time point as well as subjects in the QT/QTc population who received placebo. The PK/QTc Population will be used for the concentration-QTc analysis. PK/QTc Population will be defined for ITF2357 and for moxifloxacin.

12.4 Statistical Analyses

Details of statistical analyses will be developed in a SAP that will be prepared after completion of the final protocol.

12.4.1 Cardiodynamic ECG Assessment

The primary analysis will be based on concentration-QTc modeling of the relationship between plasma concentrations of ITF2357 and change-from-baseline QTcF (ΔQTcF) with the intent to exclude an effect of placebo-corrected ΔQTcF (ΔΔQTcF) >10 msec at clinically relevant plasma levels. In addition, the effects of ITF2357 on the placebo-corrected ΔQTcF, ΔHR, ΔPR, and ΔQRS (ΔΔQTcF, ΔΔHR, ΔΔPR, and ΔΔQRS) will be evaluated at each post-dosing time point ('by time point' analysis). An analysis of categorical outliers will be performed for changes in QTcF, HR, PR, QRS, T-wave morphology and U-wave presence.

Assay sensitivity will be evaluated by concentration-QTc analysis of the effect on ΔΔQTcF of moxifloxacin using a similar model as for the primary analysis. Assay sensitivity will be deemed as met if the slope of the concentration-QTc relationship/ΔQTcF is statistically significant at 10% level of significance in a 2-sided test and the predicted QT effect (i.e. the lower bound of the 2-sided 90% CI of ΔΔQTcF) is above 5 msec at the observed geometric mean C_{max} of 400 mg moxifloxacin.

General methodology:

All statistical analysis of the study will be performed using the statistical software SAS for Windows Version 9.4 or newer (SAS Institute, Inc., Cary, NC). In all calculations, zero will be substituted for concentrations below the quantification limit of the assay. Data collected from all randomized subjects will be presented in data listings. Both absolute values and change-from-baseline values for each subject will be given where applicable. All continuous data will be listed with the same precision as will be presented in the database. Data listings will be sorted by treatment, subject ID, and time point. Missing values will be represented by an empty cell and no imputation will be made.

For all descriptive statistics of continuous ECG parameters, data will be summarized including number of subjects (n), mean, median, SD, SE, 90% CI, Min, and Max by treatment and time point. For all modeling results of the by-time-point analysis of change-from-baseline values of continuous ECG parameters, n, LS mean, SE, and 90% CI will be included. Modeling results of the by-time point analysis of placebo-corrected change-from-baseline will also include LS mean, SE, and 90% CI. Mean and median values will be rounded to the nearest tenth. SD, SE, and CI will be rounded to the nearest hundredth. For the concentration-QTc analysis, 2 decimal places will be shown for all effect estimates for all results which have an absolute value greater than 0.05. Each effect estimate with an absolute value ≤ 0.05 will be displayed with 2 significant

figures. The CI of the effect estimate will display 1 more decimal place than the effect estimate. SE and *P* values will be reported with 4 digits and *P* values < 0.0001 will be reported as < 0.0001. Degrees of freedom (*df*), and t-value will be reported to the nearest tenth and nearest hundredth, respectively. Categorical data will be summarized 2 ways, by subject and by time point. Subject data will be summarized using the count of distinct subjects that fall into the category and the percentage of the total number of subjects. Time point data will be summarized using the count of the assessments that fall into the category and the percentage of the total number of assessments. Percentages will be rounded up or down to the next integer percentage. Population counts (either number of subjects or number of time points at the assessment) for each treatment group will be used as the denominator in the calculation of percentages unless otherwise specified.

Baseline:

For all continuous ECG parameters from each period, baseline will be the average of the derived ECG intervals from the 3 ECG time points (-45, -30, and -15 minutes) prior to treatment administration on Day 1 in each period for the respective period. For T-wave morphology and U-wave presence in each period, baseline includes findings observed in any of the replicates from the 3 time points prior to dosing on Day 1 in each period for the respective period.

Concentration-QTc analysis (primary analysis):

The relationship between plasma concentrations of ITF2357 and change-from-baseline QTcF (ΔQTcF) be quantified using a linear mixed-effects modeling approach. The model will include ΔQTcF as the dependent variable, plasma concentrations of ITF2357, as the explanatory variate (0 for placebo), centered baseline QTcF (i.e., baseline QTcF for individual subject minus the population mean baseline QTcF for all subjects in the same treatment period) as an additional covariate, treatment (active = 1 or placebo = 0), time (i.e., nominal post-dose time point) as fixed effects, and random effects on the intercept and slope per subject (Garnett et al. 2018).[11]

The degrees of freedom of estimates will be determined by the Kenward-Roger method. From the model, the slope (i.e., the regression parameter for concentrations of ITF2357and the treatment effect-specific intercept (defined as the difference between active and placebo) will be estimated together with the 2-sided 90% CI. The estimates for the time effect will be reported with degrees of freedom and SE.

The geometric mean of the individual C_{max} values for subjects in each of the active dose groups will be determined. The predicted effect and its 2-sided 90% CI for ΔΔQTcF at this geometric mean C_{max} will be obtained. If the upper bound of the 2-sided 90% CI (equivalent to the upper bound of the 1-sided 95% CI) of the predicted QTc effect (ΔΔQTcF) is below 10 msec at clinically relevant plasma levels, it will be concluded that ITF2357, does not cause clinically relevant QTc interval prolongation within the observed plasma concentration ranges.

To evaluate the adequacy of model fit with respect to the assumption of linearity, the observed ΔQTcF values adjusted by population time effect estimated from the model will be used. These individual placebo-adjusted ΔQTcFi,k (ΔΔQTcFi,k) values equal the observed individual ΔQTcFi,k for subject administered with ITF2357 or placebo at time point k minus the estimated population mean placebo effect at time point k (i.e., time effect). A quantile plot, i.e. plot of the

quantiles (deciles) of observed ITF2357 concentrations and the mean placebo-adjusted ΔQTcF (ΔΔQTcF) and 90% CI at the median concentration within each decile will be given. The regression line presenting the model-predicted ΔΔQTcF (as described by Tornøe et al., 2011) will be added to evaluate the fit of a linear model and visualize the concentration-response relationship.[12]For evaluation of the HR-corrected QT interval, a scatter plot and quantile plot of QTcF and RR intervals by treatment with regression line and a linear mixed-effects line (90% CI), respectively, also will be given. CCI

CCI

CCI

[redacted]

By-time point analysis (secondary analysis):

The analysis for QTcF will be based on a linear mixed-effects model with ΔQTcF as the dependent variable, period, sequence, time (i.e., nominal post-dose time point), treatment (therapeutic and supratherapeutic doses of ITF2357, 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 post-dose time points for subject within treatment period. If the model with unstructured covariance matrix fails to converge, other covariance matrix such as compound symmetry and autoregressive will be considered. The model will also include a subject-specific random effect. If the fixed effects for period and/or sequence should prove to be non-significant (that is, if the p-value >0.1), these effects may be removed from the model and the analysis will be repeated without those covariates. From this analysis, the LS mean, SE, and 2-sided 90 % CI will be calculated for the contrast "ITF2357 versus placebo" for each dose of ITF2357 at each post-dose time point, separately.

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

Categorical analysis:

The analysis results for categorical outliers will be based on treatment emergent events (i.e., new findings compared to baseline); T-wave morphology and presence of U-waves will be summarized in frequency tables with counts percentages for both number of subjects and number of timepoints. For categorical outliers, the number (percentage) of subjects as well as timepoints who had increases in absolute treatment emergent QTcF values >450 and ≤480 msec, >480 and ≤500 msec, or >500 msec, and changes from pre-dose baseline of >30 and ≤ 60 msec, or >60 msec; increase in PR from pre-dose baseline >25% to a PR>210 msec; increase in QRS from pre-dose baseline >25% to a QRS >120 msec; decrease in HR from pre-dose baseline >25% to a HR <50 bpm; and increase in HR from pre-dose baseline >25% to a HR >100 bpm will be determined. For T-wave morphology and U-wave presence, treatment-emergent changes will be assessed, i.e., changes not present at baseline. For each category of T-wave morphology and of U-waves, the category will be deemed as present if observed in any replicates at the time point.

12.4.2 Pharmacokinetic Assessment

Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (arithmetic and geometric means, SD, CV%, Min, Max, and median) of the plasma concentrations versus time will be presented as well for the pharmacokinetic parameters. No inferential statistical analysis of data is planned.

12.4.3 Safety Assessments

TEAEs will be tabulated by treatment for all subjects who received at least 1 dose of study drug (safety population). Changes from baseline values in vital signs, ECG, and clinical laboratory parameters will be evaluated and tabulated by study treatment. Safety and tolerability data will be reported using descriptive statistics. Demographic parameters will be summarized descriptively. No inferential statistical analysis of data is planned.

12.4.4 Adverse Events of Special Interest

The rates of these AESI should be compared in the treatment and control period. Subgroup analyses should be conducted in terms of age, gender, pre-existing cardiac disease, electrolyte disturbances and concomitant medications.

13. Regulatory Considerations and Quality Assurance

13.1 Independent Ethics Committee Approval of Protocol and Other Study Documents

The Qualified Investigator agrees to provide the IEC with all appropriate documents, including a copy of the protocol/amendments, ICFs, advertising text (if any), Investigator's Brochure (if any) and any other written information provided to study subjects. The trial will not begin until the IEC favourable written approvals for the above-mentioned study documents have been obtained. A written informed consent in compliance with ICH Guideline for GCP E6 (R2) and US Title 21 Code of Federal Regulations (CFR) Part 50 shall be obtained from each subject before entering the study or performing any unusual or non-routine procedure that involves risk to the subject. The original signed and dated ICF will be kept in the clinical binder at Syneos Health and a copy will be given to the subject.

In the event that the protocol is amended, the revised protocol must be approved by the IEC prior to its implementation, unless the changes involve only logistical or administrative aspects of the trial, in such cases a notification will be sent to the IEC for their information, but no approval is expected. If a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IEC, prior to subsequently obtaining each subject's consent.

The Qualified Investigator and the Sponsor's representative must sign the protocol and its amendments (if any) before initiating the study.

It is the Sponsor's responsibility to submit the protocol and its amendments (if any), and the ICFs to regulatory authorities when necessary.

Upon completion of the study, the Qualified Investigator, where applicable, should inform the institution; the Qualified Investigator/institution should provide the IRB/IEC with a summary of the study's outcome and the Sponsor and regulatory authority(ies) with any reports required.

13.2 Compliance

This study will be conducted in compliance with the protocol, GCP, and all applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IEC requirements relative to clinical studies. The study will also be conducted in compliance with the recommendations laid down in the most recent version of the Declaration of Helsinki, with the exception that registration of such Phase 1 trials in a publicly accessible database is not mandatory. As required by the Canadian regulatory agency, a Clinical Trial Application (CTA) will be submitted before the beginning of the study and a No Objection Letter (NOL) must be received prior to dosing.

13.3 Protocol Deviations

The Investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The Investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB/IEC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC for review and approval, to the Sponsor for agreement, and to the regulatory authorities, as applicable.

A significant deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the Sponsor and the IRB/IEC and agreed to by the Investigator. A significant deviation occurs when there is non-adherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject. Significant deviations can include non-adherence to inclusion or exclusion criteria, or non-adherence to ICH GCP guidelines or FDA/Competent Authorities regulations, and will lead to the subject being withdrawn from the study.

Protocol deviations will be documented by the study site staff, the investigator, and the clinical monitor throughout the course of the study. Qualified Investigator will be notified in writing by the monitor of deviations. The IRB/IEC should be notified of all protocol deviations in a timely manner, as applicable.

13.4 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under FDA 21 CFR 54. In addition, the Investigator must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

The Sponsor is not financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, the Sponsor is not financially responsible for further treatment of the subject's disease

13.5 Quality Assurance Program

Syneos Health has established Quality Control (QC) and Quality Assurance (QA) systems with written SOPs to ensure that the study will be conducted and data will be generated, recorded, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. A rigorous QC program is applied to ensure accuracy of all data and reports. QA oversees a complementary risk-based program of audits to assure compliance with applicable regulations and Syneos Health's prescriptive documentation.

13.6 Investigator Documentation

Prior to beginning the study, the Qualified Investigator will be asked to comply with ICH GCP E6 (R2) 8.2 by providing the following essential documents, including but not limited to:

- IRB/IEC approval
- US FDA 1572 Form
- Protocol signature page and Investigator Brochure originally signed by the Qualified Investigator
- Curriculum vitae for the Investigators participating in the study
- Financial disclosure information to allow the Sponsor or designee to submit complete and accurate certification or disclosure statements required under ICH GCP E6 (R2). In addition, the Investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study.
- IRB/IEC-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or legal guardian
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with GCP

13.7 Audits, Inspections and Monitoring

In accordance with the principles of GCP and GLP, the study may be inspected by regulatory authorities, the Sponsor and Syneos Health, in such case, the Qualified Investigator or designee will provide direct access to all study records. Moreover, the Sponsor is entitled to access information about the status of the study and to review the original documents of the study and to conduct study related monitoring activities.

In the event of an audit/Inspection, the Qualified Investigator agrees to allow the Sponsor, representatives of the Sponsor, or a regulatory agency (e.g., FDA or other regulatory agency) access to all study records. The Qualified Investigator or designee should promptly notify the

Sponsor of any inspection scheduled by any regulatory authorities and promptly forward copies of any inspection reports received to the Sponsor.

The study will be monitored by Sponsor's delegates according to GCP guidelines. The clinical monitor, as a representative of the Sponsor, has the obligation to follow the study closely. In doing so, the monitor will visit the Qualified Investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact. A site visit will be held prior to initiation of patient enrolment. The protocol, source documents, study drug supplies and relevant procedures will be explained to the Qualified Investigator's and his/her staff in detail at the site visit. During the study, the study monitor will visit the site regularly to check, the completeness of patient records, the adherence to the protocol and to GCP, the progress of enrolment, and also to ensure that study medication is being stored, dispensed and accounted for according to specifications. The Qualified Investigator and key trial personnel must be available to assist the monitor during these visits.

The Investigator must give the monitor access to relevant hospital or clinical records, to confirm adherence to the protocol. No information in these records about the identity of the patients will leave the study centre. Monitoring standard procedures require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs and the recording of primary efficacy and safety variables. The Investigator is responsible for completing the source documents expeditiously to capture all the relevant information, while the monitor is responsible for reviewing them and clarifying any data queries

All aspects of the study will be carefully monitored, by the Sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

14. Confidentiality and Retention of Study Records

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties. Persons to whom this study protocol is disclosed must be informed that all the information herein is confidential and may not be further divulged. These restrictions will apply as well to all future communications if deemed privileged or confidential. Publication of the study results may only be allowed with written permission from the Sponsor.

All information on a subject obtained during the conduct of the study will be kept confidential. Subjects will be identified by an anonymized identifier on all samples and study records provided to the Sponsor or designee. In compliance with ICH GCP, the Sponsor's authorized representatives, monitor(s), auditor(s), IEC, and regulatory authority(ies) will be granted direct access to the subject's original trial-related records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations. Consent from the subject for disclosure of such information will be obtained in writing in the ICF. In addition, should a subject require medical care or hospitalization during the course of the study, the clinical site may contact the treating physician with the subject's consent, except that consent may not be requested if there is an emergency situation. If the results of the study are published, the subject's identity will remain confidential.

The Sponsor and the Investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical study, in a way that ensures it is readily available and accessible, upon request, to the competent authorities. However, the medical files of subjects shall be archived in accordance with national law. After the archiving period, the Sponsor will be contacted to determine whether the study records will be forwarded to the Sponsor, destroyed or kept at the clinical site or another facility for a longer period of time at the Sponsor's expense.

The Investigator will not be allowed to publish findings from this study without prior written consent of the Sponsor.

15. References

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Annex to Amended Protocol 200148 Dated 29-SEP-2020 – Clinical Facilities

Clinical Laboratories

The following Biomedical Laboratories will be used:

CDL Laboratories
5990 Chemin de la Côte-des-neiges
Montréal (Québec), Canada, H3S 1Z5
Tel.: 1-514-344-8022

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Syneos Health
5160, boul. Décarie, suite 800
Montréal (Québec), Canada, H3X 2H9
Tel.: 1-514-485-7500

If another biomedical laboratory is used, this will be documented and annexed to the protocol.