Statistical Analysis Plan

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.

Sponsor Study No. ITF/2357/54

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Syneos Health Clinique Inc. Project No. 200148

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Sponsor Study No.: ITF/2357/54

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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 (300 mg) of ITF2357 (Givinostat) and Moxifloxacin on QT/QTc Interval in Healthy Male and Female Subjects.

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REVISION HISTORY

Version	Date	Summary of Revisions	Revised By
Final 1.0	21-SEP-2020	NA – Original version	PPD
Amendment I	26-APR-2021	See below for a description of changes	PPD

Changes included in Amendment I:

The changes from SAP Text Final 1.0 are as below:

1. Abbreviation for C_{last}, Clr, and R_{max} were added to the List of Abbreviations.
2. Study protocol updated to Protocol Amendment 1 in Section **1.**
3. Section **3.3** Treatment Description is revised and elaborated more for better clarity of treatment administration.
4. WHODRUG the most current version will be used to classify all prior and concomitant medication. This change will reflect in section **8.5**.
5. Procedure of weight assessment has been added in section **8.6** as follows:
   Three weights of the three syringes will be included in CRF and SDTM (9 weights total, for every dosing):
   - Labeled empty syringe with tip cap
   - Syringe filled with 10 mL with tip cap
   - Empty syringe with tip cap post-dose

   The actual number of tablet taken for Moxifloxacin will also be included in CRF.
   Separate listing for each treatment will be provided with total dose administered.
6. No Physical examination listing and summary will be provided. This change will reflect in section **9.1**. Also, in this section clarification has been added regarding how data on body measurements will be presented.
7. Section 9.2.1 clarifications added regarding AESI recording and how the data will be presented.
8. Section **9.3**, [PT and PTT] has been added to clarify coagulation parameters as per protocol.
9. MedDRA® the most current version will be used to classify all medical history and adverse events. This change will reflect in section **8.4** and **9.2**.
10. Triplicate ECG will be performed at screening. The averaged value of three QTc will be included in STDM. This change will be reflected in section **9.5.** Also, in this section clarification has been added regarding how the data of the ECGs will be presented.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
Ae_{0-t}	Cumulative Urinary Excretion From Time Zero to Time t
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC	Area Ander the Curve
AUC_{0-t}	Area under the concentration-time curve from time zero
AUC_{0-12}	Area under the concentration-time curve from time zero to 12 hours
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity (extrapolated)
BP	Blood Pressure
bpm	Beats per minute
CL/F	Total body clearance, calculated as Dose/AUC_{0-inf}
CI	Confidence Interval
CL	Renal Clearance
C_{last}	The last measurable concentration
Clr	Renal clearance
C_{max}	Maximum Plasma Concentration
CRF	Case Report Form
CTCAE	National Cancer Institute Common Terminology Criteria for AE
CV	Coefficient of Variation
CYP	Cytochrome P450
ECG	Electrocardiogram
EPQT	Early Precision QT analysis technique (formerly High Precision QT)
FDA	Food and Drug Administration
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HEENT	Head, Eyes, Ears, Nose, and Throat
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IC50	Concentration at Which 50% Inhibition Observed
INR	International Normalized Ratio

IV	Intravenous
K_{el}	Elimination rate constant
kg	Kilogram
L	Liter
LDL	Low-density lipoprotein
LLN	Lower Limit of Normal
LOESS	Locally weighted scatter plot smoothing
LS	Least squares
Max.	Maximum
MDMA	3,4-methylenedioxymethamphetamine
mg	Milligram
Min.	Minimum
min	Minute
mL	Milliliter
mmHg	Millimeters of Mercury
ms	Millisecond
OT	Oral temperature
OTC	Over-the-Counter
PCP	Phencyclidine
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PR	PR Interval of the ECG
PTT	Partial Thromboplastin Time
PT	Prothrombin Time/Preferred Term
Q-Q	Quantile-quantile
QRS	QRS interval of the ECG
QT	QT Interval of the ECG
QTc	Corrected QT interval
QTcF	Corrected QT interval using Fridericia's formula
R_{max}	Maximum rate of urinary excretion
RR	RR interval of the ECG
Rsq	r-squared
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SD	Standard Deviation
SE	Standard Error
SOP	Standard Operation Procedure
TEAE	Treatment-Emergent Adverse Event
TdP	Torsade de Pointes
$T_{½\ el}$	Half-Life
T_{max}	Time of Maximum Concentration
T_{Rmax}	Time of Maximal Urinary Excretion
TQT	Thorough QT
ULN	Upper Limit of Normal
Vd/F	Apparent volume of distribution, calculated as Dose/K_{el} x $AUC_{0\text{-}inf}$
WBC	White Blood Cell
Δ	Change From baseline
ΔΔ	Placebo-corrected or Placebo-adjusted Change From Baseline

1. Introduction

This analysis plan (SAP) is intended to give a detailed description of the summaries and the analyses that will be generated for the present study by Syneos Health or a designee. Analyses specified in this plan are based on Italfarmaco S.p.A., Study Protocol No. ITF/2357/54 (Amendment I) dated 29 SEP, 2020 (Syneos Project No. 200148). Cardiodynamic electrocardiogram (ECG), safety, tolerability, pharmacokinetic (PK) and pharmacodynamics (PD) analyses will all be described.

This document defines the populations to be analyzed and provides full details of the statistical analyses, data displays, and algorithms to be used for data derivations to aid in the production of the statistical output and the statistical section of the cardiac safety report in regard to electrocardiogram (ECG) and concentration-QTc analyses. Relevant subject characteristics as well as the electrocardiographic parameters that will be evaluated are described along with the specific statistical methods.

The plan may change due to unforeseen circumstances and any changes made after the plan has been finalized will be documented. If additional analyses are required to supplement the planned analyses described in the SAP, the changes and justification for the changes will be outlined in a SAP amendment and in the clinical study report (CSR). No change will be made without prior approval of the study sponsor. No revision to the SAP is required for changes that do not affect the statistical analysis methods, definitions, or rules defined in this document.

When applicable, all methodology and related processes will be conducted according to Syneos' standard operating procedures (SOPs) as appropriate. Protocol deviations occurring during the study will be listed.

Shells for all statistical tables, figures and listings referred to in this SAP will be displayed in a separate document.

2. Study Objectives

2.1 Primary Objective

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

  The evaluation will be done on the QTc interval corrected for heart rate (HR) using the Fridericia method (QTcF).

2.2 Secondary Objectives

- To evaluate the effect of a single oral therapeutic (T) and a supratherapeutic dose (ST) of ITF2537 on other ECG parameters: heart rate (HR), PR interval, 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.

3. Study Design

3.1 General Design

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

3.2 Study Procedures

The study will comprise of a Screening visit, Day -1 (check-in), study Day 1 through to Day 4, Study Exit /Early Termination, and a Follow-up call (Day 12 ± 2 days). Each of the 4 periods will be separated by a washout of 7 days or more. The duration of subject participation, including screening period, for each subject should last approximately 8 weeks.

The overall schedule of procedures and assessments is provided in the protocol.

3.3 Treatment Description

Treatment T – Therapeutic dose of ITF2357 100 mg (ITF2357 10 mg/mL oral suspension – 140 mL/bottle and Placebo oral suspension – 140 mL/bottle).

Treatment T will be administered as 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), it 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. The administration will be done under fasting conditions.

Treatment ST – Supratherapeutic dose of ITF2357 300 mg (ITF2357 10 mg/mL oral suspension – 140 mL/bottle).

Treatment ST will be administered as 3 x 10 mL of ITF2357 10 mg/mL oral suspension (30 mL in total, for a total ITF2357 dose of 300 mg), it 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. The administration will be done under fasting conditions.

Treatment P – Placebo (Placebo oral suspension – 140 mL/bottle).

Treatment P will be administered as 3 x 10 mL of the matching placebo oral suspension (30 mL in total), it 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. The administration will be done under fasting conditions.

For Treatments T, ST, and P, the study medication will be prepared within 1 hour of dosing. Additionally, subjects will drink all suspension immediately (within 2 minutes) after the contents of the 3 syringes is transferred to the same glass.

Treatment M – Moxifloxacin (Positive Control, Moxifloxacin hydrochloride 400 mg tablet).

Treatment M will be administered on an open label basis, as 1 x 400 mg tablet under fasting conditions with 240 mL of water.

3.4 Confinement 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.

3.5 Sample Size

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

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3.6 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 sequences, described in Table 1 below:

Table 1 Study Treatment Sequence

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, and will leave the pharmacy 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.

3.7 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 (prothrombin time [PT] and partial thromboplastin time [PTT]), and urinalysis results will be reviewed by the 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.

4. Change From the Protocol

No changes in planned analyses were done compared to the protocol.

5. Primary and Secondary Parameters

The primary and secondary parameters for the study are summarized as:

Primary parameters:

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

Secondary parameters:

- 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
- Plasma and urine PK parameters for ITF2357, ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide, and moxifloxacin:
  Parameters for plasma PK: $AUC_{0\text{-}t}$, $AUC_{0\text{-}12}$, $AUC_{0\text{-}inf}$, Residual area, C_{max}, T_{max}, $T_{½\ el}$, and K_{el}, CL/F, and V_d/F;
  Parameters for urine PK: $Ae_{0\text{-}t}$, R_{max}, T_{Rmax}, and Clr.
- Parameters for Safety and tolerability include 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.

6. Analysis Populations

Study populations will include: Safety population, QT/QTc population, Pharmacokinetic population, Pharmacokinetic concentration population and PK/QTc population.

When determining data availability for the study populations, the following aspects are to be considered (but not to be limited to) : inclusion and exclusion criteria, acceptable times for visit dates and measurements, compliance with treatment, the nature and quality of the data, withdrawal and any protocol deviation. Any decision for excluding data from the final data set will be provided with a detailed explanation and will be properly recorded and dated with Sponsor's approval. The final responsibility of deciding which subjects are to be included or excluded lies with the Investigator, and/or the Sponsor.

The analysis of safety and tolerability parameters will be based on the safety population detailed in Section 6.1 below. The analysis of cardiodynamic ECG parameters will be based the QT/QTc population detailed in Section 6.2 below. The analysis of PK parameters will be based on the PK population detailed in Section 6.3 below. PT/QTc population will be detailed in Section 6.4 below.

6.1 Safety Population

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

6.2 QT/QTc Population

The QT/QTc Population will be defined as 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.

6.3 Pharmacokinetic Population

The PK population will de defined as all subjects completing at least 3 periods, including at least Treatments T, ST, and M and for whom the PK profile can be adequately characterized. The PK population will be used for calculation of PK parameters and statistical 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 may be evaluated after completion of the PK 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.

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

Pharmacokinetic Concentration Population

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

6.4 PK/QTc Population

The PK/QTc Population will be defined as 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.

7. Interim Analyses

No formal interim analysis is planned.

However, a preliminary analysis using PK data will be done after the bioanalytical phase is completed. The PK parameters will be calculated using nominal sampling times as actual sampling times will not be available for these preliminary analyses. Only descriptive statistics of PK parameters will be calculated and provided.

8. Study Population and Exposure

No inferential analysis will be done. Only observed data will be used.

8.1 Subject Disposition

Subject disposition will be summarized by treatment and overall (frequency and the percentage of subjects). Subject completion and discontinuation information will be listed. In addition, subjects who were dismissed from a period or who did not complete a dosing period will also be presented in this listing, including absence/early discontinuation reason, date and time of discontinuation.

8.2 Protocol Deviations

The protocol deviations will be summarized by the categorized deviations and listed by subject, for the safety population.

8.3 Demographics and Baseline Characteristics

The descriptive statistics (mean, median, standard deviation [SD], minimum [Min], maximum [Max], and sample size) will be calculated for continuous variables (age, body weight, height and body mass index [BMI]) considering last results (scheduled or unscheduled) obtained at screening. Frequency counts and percentages will be tabulated for categorical variables (age group, sex, ethnicity, and race). Results will be presented summarized by treatment and overall for the safety population (overall) and for PK population. All demographic characteristics will be listed by subject.

8.4 Medical History

Medical history at screening will be listed by subject and summarized by treatment group for the safety population. The Medical Dictionary for Regulatory Activities (MedDRA®)will be used to classify all medical history findings by System Organ Class (SOC) and Preferred Term (PT). The MedDRA dictionaries used on the study will be updated to the current versions every 6 months to coincide with the dictionary updates cycles.

8.5 Prior and Concomitant Medications

The use of prior and/or concomitant medication will be monitored throughout the study and listed by subject for the safety population. The World Health Organization Drug Dictionary will be used to classify all medication reported as from screening through study exit/early termination. The most current version will be used throughout the trial.

Prior and concomitant medications will be listed and the Anatomical Therapeutic Code (ATC) will be included.

8.6 Study Drug Administration

The study drug administration details (including sequence, period, treatment received, total dose administered, start and end date/time of administration) will be listed by subject and by treatment for the safety population.

In order to calculate treatment compliance, three weights of the three syringes will be included in CRF and SDTM (9 weights total, for every dosing):
- Labeled empty syringe with tip cap
- Labeled syringe filled with 10 mL with tip cap
- Labeled empty syringe with tip cap post-dose

The actual number of tablets taken for Moifoxacin will also be included in CRF. Separate listing for each treatment will be provided with total dose administered and compliance calculation.

9. Safety Analyses

Safety data will be evaluated for the safety population through the assessment of AEs, AESIs, laboratory parameters (hematology, biochemistry, coagulation, and urinalysis), safety ECG, clinical signs and symptoms from physical examination, and vital signs assessments. Treatment-Emergent Adverse Events (TEAEs), laboratory values, and vital signs, will be summarized by treatment and overall, changes from baseline values in vital signs, ECG and clinical laboratory parameters will be evaluated and tabulated. Safety and tolerability data will be reported using descriptive statistics, it will be listed or summarized but will not be subjected to inferential analysis.

Schedule 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. Results from repeated / unscheduled tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

9.1 Physical Examination Findings

A complete physical examination will be performed at screening and at study exit. For Periods 1, 2, and 3, a brief physical examination will be performed at check-in (Day -1), and approximately 24 (Day 2), and 72 (Day 4) hours post dose. For Period 4, a brief physical examination will be performed at check-in (Day -1), and 24 hours post dose.

The 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.

The 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. The data of body measurements for screening are included in the demographic data listing of the SAP, while for the other visits only weight is collected and this information is included in the CRF.

Any abnormal findings judged to be clinically significant (CS) will be documented as medical history or as an AE, depending upon time of observation whether noted at screening, prior to dosing or after dosing, as appropriate.

9.2 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, and they will be followed-up until complete resolution, or until the Investigator judges it to be safe to discontinue follow-up.

Treatment-emergent AEs and non-TEAEs (those occurring prior to administration of study medication or that first occurred prior to study drug administration and did not worsen in frequency or severity) will be listed. TEAEs will be defined as AEs that occur on or after the date and time of study drug administration, or those that first occur pre-dose but worsen by increase in occurrence or severity after study drug administration.

The incidence of TEAEs will be summarized using the safety population. The updated version of MedDRA® dictionary will be used to classify all AEs reported during the study by SOC and PT.

Incidence of subjects who experienced TEAEs will be presented by treatment and overall, SOC, PT, by Investigator-assessed relationship and also by severity. Each subject may only contribute once to each of the incidence rates, for a TEAE following a given treatment, regardless of the number of occurrences; the highest severity or highest relationship will be presented, as appropriate. In each table, SOC will be presented in descending order of overall incidence rate in terms of frequency of subjects and then in frequency of events (alphabetical order will be used in case of equal rates). For each SOC, PT will be presented the same way.

Incidence of TEAEs (number of events) will also be presented by treatment and overall, by SOC, and PT, by Investigator-assessed relationship and severity.

Frequency of subjects experiencing Serious Treatment Emergent Adverse Events and Suspected Unexpected Serious Adverse Drug Reactions; and the number of events would be also summarized per treatment and overall. Also, these events and Serious Adverse Events will be listed per subject.

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

The relationship of an AE or SAE to the study drug will be assessed according to the study protocol 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.

9.2.1 Adverse Events of Special Interest

Adverse events of special interest (AESIs) for this study will 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. When these events are identified they should be examined closely for other risks factors, and evaluated. Also the need for evaluation by a cardiac specialist will be discussed by the Investigator and the Sponsor.

Incidence of AESIs (number of events) will also be presented by treatment and overall for severity and relationship and by treatment and overall for age, gender, pre-existing cardiac disease, electrolyte disturbances and concomitant medications.

9.3 Laboratory Parameters

Clinical laboratory (hematology, biochemistry, coagulation [PT and PTT], and urinalysis) results will be obtained 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.

Hematology parameters include the following: complete blood count with differential, hemoglobin, and hematocrit.

Biochemistry parameters include the following: cholesterol (total, low-density lipoprotein [LDL] and high-density lipoprotein [HDL]), triglycerides, albumin, alkaline phosphatase, aspartate aminotransferase [AST], alanine aminotransferase [ALT], urea, cystatin C, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, and total protein.

Coagulation parameters include the following: PT and PTT.

Urinalysis parameters include the following: 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.

Human Immunodeficiency Virus (HIV) antigen and antibody, Hepatitis B surface antigen (HBsAg), and Hepatitis C (HCV) antibody detection will be performed at screening.

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.

A urine drug screen (amphetamines, methamphetamines, barbiturates, benzodiazepines, tetrahydrocannabinol, cocaine, opiates, phencyclidine [PCP], 3,4-methylenedioxy-methamphetamine [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.

Listings of all clinical laboratory results will be provided with the abnormal values flagged with "L" (below normal) and "H" (above normal) for continuous parameters, and “A” (abnormal) for categorical parameters, including the evaluation of the abnormal result as clinically significant or not clinically significant.

Clinically significant laboratory abnormalities will be recorded as AEs or SAEs and identified as Grade 3 to 4 laboratory test results graded according to numeric laboratory test criteria in the latest version of Common Technical Criteria for Adverse Events (CTCAE version 5.0) if available, otherwise according to the latest version of Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Corrected will be provided.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) for each clinical laboratory test (continuous variables) will be presented by treatment and overall. Change from baseline for hematology, biochemistry, coagulation, and urinalysis will be presented. Baseline is defined as the last assessment prior to dosing with study drug in that same treatment period (i.e. baseline measurements must have been collected prior to administration of study drug in that treatment period). The unscheduled results will not be included in the summary tables. The repeated laboratory test performed due to safety reasons, after a clinically significant abnormal results is obtained, will be considered as follow-up and analyzed as such. For categorical variable (urinalysis test), the number of subjects (frequency and percentage) will be tabulated by results (e.g., negative, positive, trace …). A summary table of shifts from baseline to study exit will be provided. Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

9.4 Vital Signs

Blood pressure (BP), heart rate (HR), respiratory rate (RR), and oral temperature (OT) will be measured in a supine position at: screening, pre-dose, and approximately 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, 2 measures performed after at least 5 minutes of resting in supine position should be taken 2 minutes apart from each other.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) will be presented overall for baseline and study exit and by the associated current treatment for on-study measurements and for each vital sign measurement. Descriptive statistics for change from baseline to study exit for on-study measurements will also be presented. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to drug administration in each period. Unscheduled results will not be included in the summary tables otherwise. Results from post-dose repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A table summarizing the descriptive statistics, a table showing shift of results (low, normal and high) from baseline to post dose and a listing of all vital signs results will be provided.

9.5 12-Lead Safety ECG

Supine 12-lead ECG will be performed at screening (triplicate ECG will be performed at screening. The averaged value of three QTc will be included in STDM), 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 prioritized over safety and other PK assessments

The standard ECG parameters will be measured, including ventricular heart rate (VR), PR interval, QRS interval, QT interval, QTcB interval (Bazett's formula correction), and QTcF interval (Fridericia's formula correction). The QTcF interval will be used for clinical evaluations.

A table summarizing the descriptive statistics, a table showing shift of results (normal, abnormal non-significant (NCS) and abnormal clinically significant (CS)) from baseline to post dose and a listing of all ECG results will be presented.

10. Cardiodynamic ECG Assessment

10.1 ECG and Pharmacokinetic Sample Collection

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

Continuous 12-lead ECG recordings will be performed from 1 hour prior to dosing in each treatment period to 36 hours post-dose. 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.

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.

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) Ml2R 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 the study protocol, and will be measured centrally by ERT.

ECG intervals will be measured by the core laboratory in a blinded manner using the Early Precision QT technique (EPQT) (see Appendix A for more details). The ECG database will be locked before any statistical analysis is undertaken.

Blood samples for PK determination will be drawn at the same time points in each period (i.e., 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) immediately after the ECG extractions and additionally at 48, 60, and 72 hours post-dose. ITF2357 plasma levels will be of interest to correlate with pharmacodynamic 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.

10.2 Statistical Methods

10.2.1 General Methodology

All statistical analyses will be performed using the statistical software SAS® for Windows Version 9.4 or higher (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 (i.e., HR, PR, QRS, and QTcF), data will be summarized including number of subjects (n), mean, median, standard deviation (SD), standard error (SE), 90% confidence interval (CI), minimum, and maximum 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, least squares (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

10.2.2 Baseline

For all continuous ECG parameters from each period, baseline is defined as the average of the measured ECG intervals from the 3 ECG time points (−45, −30, and −15 minutes) prior to treatment administration on Day 1 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 for the respective period.

10.2.3 QT Correction Methods

The QT and RR value for each beat will be used for HR correction.

The Fridericia's correction (QTcF) is defined as QTcF (ms) = QT (ms)/[RR(ms)/1000]1/3.

For evaluation of the HR-corrected QT interval, a scatter plot and quantile plot of QTcF and RR intervals by treatment with a regression line and a linear mixed-effects line (90% CI), respectively, also will be given.

10.3 Analysis

10.3.1 Concentration-QTc Analysis (Primary Analysis)

The relationship between plasma concentrations of ITF2357 and ΔQTcF will be quantified using a linear mixed-effects modeling approach. The model will include ΔQTcF as the dependent variable, plasma concentration 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 within 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 intercept and slope per subject (Garnett et al).

An unstructured covariance matrix will be specified for the random effects. If convergence cannot be achieved even after appropriate rescaling of the concentrations, the random effect on the slope and intercept will be dropped, in this order, until convergence is achieved. The degrees of freedom (*df*) estimates will be determined by the Kenward-Roger method. From the model, the slope (i.e., the regression parameter for concentration ITF2357) and 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 effects will be reported with *df* 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 i.e., slope estimate × concentration + treatment effect-specific intercept) 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 ms at clinically relevant plasma levels, it will be concluded that ITF2357 does not cause clinically relevant QTc 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 $\Delta QTcF_{i,k}$ ($\Delta\Delta QTc_{i,k}$) values equal the observed individual $\Delta QTcF_{i,k}$ 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) will be added to evaluate the fit of a linear model and visualize the concentration-response relationship. CCI [redacted]

The SAS code for the concentration-QTc analysis is as follows.

```
PROC MIXED DATA=PKPD method=reml;
CLASS SUBJID TIME;
```

MODEL DQTc=TRT CONC TIME CBASE/ solution cl noint alpha=0.1 alphap=0.1 COVB DDFM=KR;
RANDOM INT CONC /type=UN SUBJECT=SUBJID s;
RUN;

Where PKPD = PK/QTc population, SUBJID = subject number, TRT = treatment (active = 1 or placebo = 0), TIME = nominal post-dose time point, CONC = plasma concentration of ITF2357, CBASE = centered baseline QTcF, and DQTc = ΔQTcF.

Note: ESTIMATE statements will be included for the prediction of the effect in each of the active dose groups.

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10.4 Assay Sensitivity

The analysis to show assay sensitivity will be also based on the concentration-QTc analysis of the effect on ΔΔQTcF of 400 mg oral moxifloxacin using a similar model as for the primary analysis. That is, the relationship between moxifloxacin plasma concentration and ΔQTcF will be investigated by linear mixed-effects modeling. The model will include ΔQTcF as the dependent variable, moxifloxacin plasma concentration as the explanatory variable (0 for placebo), centered baseline QTcF (i.e., baseline QTcF for individual subject at each post-dose time point minus the population mean baseline QTcF for all subjects in the same treatment period) as an additional covariate, treatment (moxifloxacin = 1 or placebo = 0) and time (i.e., post-dose time point) as fixed effects, and a random intercept and slope per subject (Garnett et al). The geometric mean of the individual C_{max} values for subjects receiving the single dose of 400 mg moxifloxacin will be determined. The predicted effect and its 2-sided 90% CI for ΔΔQTcF (i.e., slope estimate × concentration + treatment effect-specific intercept) at this geometric mean C_{max} will be obtained.

If the slope of the concentration-QTc (ΔQTcF) for moxifloxacin is statistically significant at 10% level for 2-sided test and the lower bound of the 2-sided 90% CI of the predicted effect of ΔΔQTcF is above 5 ms at the geometric mean C_{max} for 400 mg moxifloxacin, assay sensitivity will be deemed to have been demonstrated.

10.5 By-Time Point Analysis

The by-time point 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 dose of ITF2357, supratherapeutic dose 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 an unstructured covariance matrix fails to converge, other covariance matrices 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 not significant (i.e., if the P value > 0.1), these effects may be removed from the model and the analysis 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" (ΔΔQTcF) for each dose of ITF2357 at each post-dose time point, separately.

For HR, PR, and QRS interval, 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 tables and graphically displayed.

The SAS code for the by-time point analysis for QTcF is as follows.

```
PROC MIXED DATA=ECG;
CLASS SUBJID TREAT TIME PERIOD SEQUENCE;
MODEL DQTc=BASE TREAT TIME TREAT*TIME PERIOD SEQUENCE/DDFM=KR;
random intercept / SUBJECT =SUBJID type=UN;
REPEATED TIME / SUBJECT = PERIOD*SUBJID type = un;
LSMEANS TREAT*TIME/CL DIFF ALPHA=0.1;
RUN;
```

Where ECG = QT/QTc population, SUBJID = subject identifier, TREAT = treatment (therapeutic dose of ITF2357, supratherapeutic dose of ITF2357, moxifloxacin, and placebo), TIME = nominal post-dose time point, BASE = baseline QTcF, PERIOD = period, SEQUENCE = sequence, and DQTc = ΔQTcF.

10.6 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 and percentages for both number of subjects and number of time points. 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 time points at which the assessments fall into the category and the percentage of the total number of time points at which assessments are performed. Counts (either number of subjects or number of time points) for each treatment group will be used as the denominator in the calculation of percentages unless otherwise specified.

A subject or time point will be determined as an outlier if the following criteria (which are assessed separately) are met for the ECG intervals (Table 2).

Table 2 Criteria for determining a subject or time point outlier

ECG interval	Categorical outlier criteria
QTcF	Treatment-emergent value of > 450 and ≤ 480 ms when not present at baseline (new onset)
	Treatment-emergent value of > 480 and ≤ 500 ms when not present at baseline (new onset)
	Treatment-emergent value of > 500 ms when not present at baseline (new onset)
	Increase of QTcF from baseline of > 30 and ≤ 60 ms
	Increase of QTcF from baseline > 60 ms
PR	Increase of PR from baseline > 25% resulting in PR > 210 ms
QRS	Increase of QRS from baseline > 25% resulting in QRS > 120 ms
HR	Decrease of HR from baseline >25% resulting in HR < 50 bpm
	Increase of HR from baseline >25% resulting in HR > 100 bpm

All outliers will be summarized for each treatment group on the basis of incidence rates. A subject will be counted only once for a particular outlier event if the subject experiences more than 1 episode of that event. The total number of time points will be based on the number of observed time points across all subjects within a treatment group.

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.

The T-wave morphology and U-wave presence categories are described as follows (Table 3).

Table 3 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.
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.

10.7 Terminology and Definitions: Placebo-corrected ΔQTcF and Placebo-adjusted ΔQTcF (ΔΔQTcF)

Change-from-baseline QTcF (ΔQTcF) will be used as the dependent variable in the concentration-QTc analysis and in the by-time point analysis.

By-time point analysis

Placebo-corrected ΔQTcF (ΔΔQTcF)

- In the by-time point analysis on the QTcF interval, LS mean, SE, and 2-sided 90% CI of ΔQTcF and ΔΔQTcF will be calculated for each active dose group and moxifloxacin group, as well as on placebo group for ΔQTcF at each post-dose time point.

Concentration-QTc analysis

Placebo-corrected ΔQTcF (ΔΔQTcF)

- In the concentration-QTc analysis, the term placebo-corrected ΔQTcF (ΔΔQTcF) will be used for the model-predicted effect across concentrations on a population level.

- *Definition*: Model-predicted mean ΔQTcF in each active dose group minus model-predicted mean ΔQTcF in the placebo group, which equals slope estimate × concentration + treatment effect-specific intercept.

Placebo-adjusted ΔQTcF (ΔΔQTcF)

- In the concentration-QTc analysis, the term placebo-adjusted ΔQTcF (ΔΔQTcF) will be used to illustrate the underlying data on both subject and population levels.
- *Definition for the estimated placebo-adjusted ΔQTcF on a subject level:* observed ΔQTcF for each subject (on active dose group or moxifloxacin group or placebo group) minus the estimated time effect (i.e., the model-predicted mean ΔQTcF in the placebo group).

- *Definition for the estimated placebo-adjusted ΔQTcF term on a population level:* the average of individually estimated placebo-adjusted ΔQTcF values at the associated median plasma concentration within each concentration decile.

10.8 Sample Size Considerations for Assay Sensitivity

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11. Pharmacokinetic Analyses

11.1 Handling of the Below the Lower Limit of Quantitation (BLQ) and the No Reportable Concentration Values

During PK and statistical analyses, drug concentrations BLQ of an assay will be considered as zero except when they occur between two non-BLQ concentrations where they will be considered as missing during PK calculations and estimations. A sample with a no reportable value occurring prior to the dosing for a given period will be replaced by zero. For tabulation, graphical representation and calculation purposes, all samples with no reportable value observed after dosing will be set to missing.

11.2 Handling of the Difference Between the Scheduled and the Actual Sampling Times

The actual clock time for dosing and the actual clock time for each collection time for the PK samples will be recorded using the electronic data capture. For all sampling times, the actual sampling times will be calculated as the difference between the sample collection actual clock time and the actual clock time of dosing. The actual post-dose sampling times expressed in hours and rounded off to three decimal digits will be used to calculate the PK parameters, except for pre-dose samples occurring prior to dosing, which will always be reported as zero (0.000), regardless of the time difference. In the PK section of the report, scheduled sampling times will be presented in concentration tables and mean graphs while actual times are presented for the individual graphs. A listing of the actual times for PKs will be provided for PK samples.

11.3 Pharmacokinetic Parameters

11.3.1 Plasma Pharmacokinetic Parameters

For PK analysis of ITF2357 and its metabolites (ITF2374, ITF2375, ITF2440, ITF2563, ITF2955 glucuronide), and moxifloxacin for each period, a total of 19 blood samples will be collected from each subject at pre-dose (baseline) and 0.500, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 7.00, 8.00, 12.0, 24.0, 36.0, 48.0, 60.0, and 72.0 hours post-dose.

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.

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. 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 analysis. 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 using a similar analysis method.

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.

Plasma concentrations for ITF2357 and its metabolites, and moxifloxacin will be used to calculate the following PK parameters by standard non compartmental methods:

AUC_{0-t}	Area under the concentration-time curve from time zero to the last measurable concentration, calculated using the trapezoidal method.
AUC_{0-12}	Area under the concentration-time curve from time zero to 12 hours, calculated using the trapezoidal method.
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity (extrapolated), calculated as $AUC_{0-t} + C_{last}/K_{el}$, where C_{last} is the last measurable concentration.
C_{max}	Maximum observed plasma concentration.
Residual area	Residual area, calculated as $100*(1- AUC_{0-t} / AUC_{0-inf})$.
T_{max}	Time of observed C_{max}.
$T_{½\ el}$	Elimination half-life, calculated as $ln(2)/K_{el}$.
K_{el}	Elimination rate constant. This parameter will be the negative of the estimated slope of the linear regression of the ln-transformed concentration versus time profile in the terminal elimination phase. Best fit method will be used to calculate the K_{el} from at least 3 concentration data points excluding the C_{max}. Rsq adjusted, the goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of Kel must be ≥ 0.8. If the Kel cannot be measured (e.g.: fewer than 3 non-zero concentrations in the terminal elimination phase or Rsq adjusted < 0.8), the PK parameters derived from K_{el} will not be reported for that individual PK profile (AUC_{0-inf}, Residual area, and T½ el). The timepoint where ln-linear K_{el} calculation begins ($K_{el\ Lower}$) and the actual sampling time of the last measurable concentration used to estimate the K_{el} ($K_{el\ Upper}$), as well as the Rsq adjusted for the ln-linear regression for the calculation of the elimination rate constant will be reported.
CL/F	Apparent total body clearance, calculated as $Dose/AUC_{0-inf}$.
V_d/F	Apparent volume of distribution, calculated as $Dose/K_{el} \times AUC_{0-inf}$.

11.3.2 Urine Pharmacokinetic Parameters

Urine samples will be collected in all subjects at 5 times or time intervals during treatment with Treatments T, ST, and P: spot pre-dose (within 2 hours before dosing), 0.00 -8.00 hour, 8.00-24.0 hours, 24.0-48.0 hours, and 48.0-72.0 hours post-dose. Before starting analysis, the biostatistician will randomly select 12 evaluable subjects for whom urine and blood samples for metabolite analysis will be analyzed; the rest of the samples will be kept frozen and analyzed similarly later on if after obtaining bioanalysis results, further analysis will be needed.

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

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

Some PK parameters may not be calculated for all or some subjects, at the discretion of the Syneos pharmacokineticist, if the concentration data is not deemed to be amenable to evaluation. Explanations for PK parameters that could not be estimated will be provided in the report.

11.4 Statistical Analyses

Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales for ITF2357 and its metabolites, and moxifloxacin. 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 PK parameters, except T_{max}, and $T_{½\ el}$.

The T_{max} and $T_{½\ el}$ data will be summarized by treatment with number of observations, mean, SD, CV, Min, median, and Max.

Whenever a PK parameter can be calculated for only one period for a subject, the subject will be excluded from the statistical analysis involving this parameter. However, data from the available period will be included in the descriptive statistics.

Additional PK statistical analyses may be performed as warranted.

12. Percentages and Decimal Places

If not otherwise specified, the following rules will be applied, with the exception of PK tables and listings described below:

Percentages will be presented to one decimal point.

Percentages equal to 0 or 100 will be presented as such without a decimal point.

Minimum and maximum will be presented with the same precision as the original values and, mean, standard deviation, and median will be presented with one more decimal place than the original values.

All digits will be used for pharmacokinetic and statistical PK calculations. For PK tables and listings, the final reportable results or data will be presented by rounding off to two decimal digits, except for the following situations (this applies to individual data and descriptive statistics):

K_{el} and Rsq adjusted data: rounded off to four decimal digits.

Pharmacokinetic parameters related to time such as T_{max}, must be reported with the same precision as the actual sampling time: rounded off to 3 decimal digits.

Concentration versus time data, as well as C_{max}: reported as they appear in corresponding dataset.

13. Handling of Missing Data

For safety,

- If an AE is recorded with an onset date corresponding to a dosing day, but the time is missing, then the AE will be assigned to the treatment phase planned treatment with a dosing planned that day.

- If an AE is recorded with an onset date that does not correspond to a dosing day, but the time is missing, then the AE will be assigned to the treatment phase planned treatment that covers the AE onset day.

- If an AE is recorded with an onset date where day and time are both missing, then the AE allocation to a treatment phase planned treatment will be done on a case by case basis considering available information (e.g. AE onset date, AE end date, AE comments, subject disposition).

For PK, only observed data will be used in the data analysis except for concentration values BLQ as described in Section 11.3. No attempt will be made to extrapolate or interpolate estimates for missing data.

For Holter, 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, as described in section 10.2.1.

14. Data Handling

The PK plasma concentrations, safety, and tolerability data will be received as SAS® datasets from the Syneos data management facility. Screening failures and ineligible volunteer's data (subject disposition) will be received from the clinical site as source data.

15. Software to be Used

PK analysis will be performed using Phoenix WinNonlin® version 8.0 or higher, which is validated for bioequivalence/bioavailability studies by inVentiv. The safety data tables and listings, as well as PK tables and listings will be created using SAS®, release 9.2 or a higher version. PK figures will be created using R version 3.5 (or higher). The CSR will be created using Microsoft® Office Word 2010, or a higher version.

16. Appendix A: Early Precision QT Analysis

Twelve-lead ECGs will be extracted in up to 10 replicates from each nominal time point prespecified in the protocol. The median value of each parameter from the set of evaluable beats in each extracted replicate will be calculated, and then the mean of all available medians (minimum 3 medians) from the nominal time point will be used as the subject's reportable value at that time point.

Early Precision QT analysis (formerly High Precision QT analysis) will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates (1 replicate consists of one 14 second ECG). Statistical quality control procedures will be 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.

Placement of fiducials and measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates will be performed using the iCOMPAS software. All beats that are deemed "high confidence" will not be reviewed by an ERT cardiac safety specialist. All low confidence beats will be reviewed manually by an ERT cardiac safety specialist and adjudicated using pass-fail criteria. The beats found acceptable by manual review will be included in the analysis. The beats confirmed to meet fail criteria will not be included in the analysis.

For the purpose of measuring PR and QRS intervals and to assess T-wave morphology and presence of U-waves, the TQT Plus algorithm will select the 3 ECG replicates with the highest quality score from the ECG extraction window. These 3 ECGs will be analyzed using a semi-automated process to determine these parameters. If 3 consecutive usable beats cannot be identified in at least 2 of the 3 replicates, then all beats in all replicates will be reviewed for that time point using a manual analysis.

If manual analysis is required, then all beats in a minimum of 3 replicates will be reviewed using the iCOMPAS software. The ERT cardiac safety specialist will review all usable beats in Lead II (or an alternate lead) for each replicate and will review and/or adjust the fiducial placements (onset of P, onset of Q, offset of S, and offset of T-wave that were electronically marked) of each waveform and also document the T wave morphology and the presence of U-waves for each beat. A replicate will only be reported if it has 3 approved, usable beats.

17. Reference List

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